Departement für Pferde, Klinik für Pferdemedizin
der Vetsuisse-Fakultät Universität Zürich

Vorsteher: Prof. Dr. med. vet. Colin Schwarzwald

Arbeit unter wissenschaftlicher Betreuung von
PD Dr. med. vet. Angelika Schoster

Investigation of the antibody response and nasal shedding after EHV-1/4 vaccination and nasal shedding of gamma herpesviruses

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Laura Scheurer

Tierärztin
von Schnottwil, Solothurn

genehmigt auf Antrag von

PD Dr. med. vet. Angelika Schoster, Referentin
PD Dr. med. vet. Claudia Bachofen, Korreferentin

2020

Content

Vetsuisse Fakultät, Universität Zürich (2020)

Med. vet. Laura Scheurer

Departement für Pferde, Klinik für Pferdemedizin
pferdemedizin@vetclinics.uzh.ch

Untersuchung der Antikörper Antwort und Nasenausscheidung nach EHV-1/4 Impfung und Nasenausscheidung von Gammaherpesviren

Zusammenfassung

Im Teil A wurden die Antikörperkinetik und Virusausscheidung nach Impfung mit einer inaktivierten EHV-1/4 Vakzine untersucht. Von 20 Pferden wurden 15 geimpft, wobei 5 Kontrollpferde ohne Impfung belassen wurden. Serumproben wurden vor jeder Impfung und monatlich während eines Jahres mittels ELISA analysiert. Nasentupfer wurden vor jeder Impfung und an den 5 folgenden Tagen mittels Real-Time PCR untersucht.
Alle Pferde waren zu jedem Zeitpunkt positiv für EHV-4. Von l5 geimpften Pferden waren 8 mindestens zu einem Messzeitpunkt und 5 über mehrere Monate EHV-1 seropositiv. Ein EHV-1 induzierter Abort ereignete sich im Stall der Kontrollgruppe, wobei 4/5 Pferde an mindestens einem Messzeitpunkt EHV-1 seropositiv waren. EHV-l/4 Virusausscheidung im Nasensekret kam nach allen Impfungen vor, am häufigsten jedoch nach der letzten Impfung im Winter.
Die humorale Immunantwort insbesondere gegen die EHV-1 Komponente der Vakzine fällt individuell und moderat aus. Die erhöhte EHV-1/4 Virusausscheidung nach der Winter Impfung könnte saisonal begründet sein.
Im Teil B wurde die Prävalenz von equinen Herpesviren (EHV) im Nasensekret von 68 zufällig ausgewählten Proben aus der Schweizer Pferdepopulation mittels Panherpes nested PCR untersucht. Von 68 Proben waren 40 positiv für ein EHV, wovon 26 auf EHV-2, 8 auf EHV-5 und 6 auf AHV-5 getestet wurden.
Wie erwartet ist die EHV-2 Virusausscheidung im Nasensekret gesunder Pferde in der Schweiz häufig, wobei EHV-5 und AHV-5 selten detektiert wurden.

Schlüsselwörter: Herpesvirus; Pferd, Vakzine, Antikörper, Virusausscheidung

Vetsuisse Faculty, University of Zurich (2020)

Med. Vet. Laura Scheurer

Department for horses, Clinic for Equine Medicine
pferdemedizin@vetclinics.uzh.ch

Investigation of the antibody response and nasal shedding after EHV-l14 vaccination and nasal shedding of gamma herpesviruses

Abstract

In Part A of this study, the antibody kinetics and equine herpesvirus (EHV) shedding following vaccination with a combined inactive whole virus vaccine were assessed. Fifteen horses were vaccinated, 5 control horses remained unvaccinated. Blood samples taken before every vaccination and monthly for one year were analyzed with an ELISA. Nasal swabs collected from every horse before vaccination and daily for 5 days were analyzed using real-time PCR.
All horses were seropositive for EHV-4 at every timepoint. Eight of fifteen vaccinated horses were seropositive for EHV-1 at least at one sampling timepoint, 5/15 displayed a sustained high antibody concentration over several months. EHV-1 abortion occurred in the barn of the control group, 4/5horses were seropositive for EHV-1 at least one sampling timepoint. EHV-1/l4 viral shedding after vaccination occurred occasionally with the highest frequency after the last administered vaccination in winter.
An individual but moderate humoral immune response is suggested. A seasonal component of EHV shedding is indicated.
In Part B of the study, the prevalence of EHV in nasal secretion of 68 randomized selected samples of the Swiss horse population were analyzed by panherpes nested PCR. Fourty of sixty-eight nasal swabs were tested positive for EHV, 26 for EHV-2,8 for EHV-5 and 6 for AHV-5.
As expected, EHV-2 viral shedding in healthy horses of Switzerland is common, whereby EHV-5 and AHV-5 is rarely detected in the investigated population.

Keywords: herpesvirus, horse, vaccine, antibody, viral shedding

Glossary

AAEP	American Association of Equine Practitioners
AHV	Asinine herpesvirus
BI	Basic immunization
BMCF	bovine malignant catarrhal fever
BoHV-1	Bovine herpesvirus 1
CF	Complement-fixing
CFT	Complement-fixing test
CG	chorionic gonadotropin
CNS	Central Nervous System
CPE	cytopathic effect
CT	cycle treshold
CTL	cytotoxic T lymphocytes
DEPC	Diethylpyrocarbonate treated water
DNA	Desoxyribonucleic acid
EHM	Equine herpes myeloencephalopathy
EHV	Equine herpesvirus
EHV-1 D_{752}	neuropathogenic EHV-1 strain
EHV-1 N_{752}	non – neuropathogenic EHV-1 strain
ELISA	Enzyme linked immunosorbent assay
EMPF	Equine multinodular pulmonary fibrosis
GHV	Gazelle herpesvirus
gB	glycoprotein B
gG	glycoprotein G
gH	glycoprotein G
gp	glycoprotein
hpi	hours post infection
HSV	herpes simplex virus
ISCOM	Immune stimulating complexes
IWVV	inactivated whole virus vaccine
MHC-1	major histocompatibility complex 1
MLV	modified live vaccine
nip	non-interpretable
nt	nucleotid
OD	optical density
ORFS	open reading frames
PBL	Peripheral blood lymphocytes
PBMC	Peripheral blood mononuclear cells
RFLP	restriction fragment length polymorphism
PRV	pseudorabies virus
SLN	submandibular lymph nodes
SN	serum neutralizing
URT	upper respiratory tract
VN	virus neutralizing
VNT	Virus Neutralization Test

1 Introduction

1.1 Herpesviruses

The order of *Herpesvirales* contains three families, three subfamilies, 17 generas and 90 species. A wide range of animals can be infected, including mammals, reptiles, birds, fish, frogs and even non-vertebrae.[1] The family of *Herpesviridae* is divided in three subfamilies: *Alpha-, Beta- and Gammaherpesvirinae*. Herpesviruses have a wide range of host species and cause various clinical signs, ranging from no signs to lethal disease. The crossing of species barriers can lead to severe disease in non-adapted species.[2] Latency in different cells, such as neurons or lymphocytes, and therefore lifelong infection is typical for herpesviruses.[2] During latency, no infectious virus is excreted, and no viral DNA can be detected. Reactivation of the virus leads to virus-shedding by entering the lytic cycle.[3]

1.1.1 Equine Herpesviruses

Currently nine equine herpesviruses, members of the subfamilies *Alpha- and Gammaherpesvirinae*, are recognized.[4] EHV-6 to 8 are also known as asinine herpesvirus (AHV, AHV-1 to 3) as they may cause diseases in donkeys. EHV-9 is also called gazelle herpesvirus (GHV) and is a pathogen of Thomson's gazelles (Tab. 1).[5-7]

Table 1 Overview of equine herpesviruses

Subfamily **Genus**	**Domestic horse** **(Equus caballus)**	**Donkey** **(Equus asinus)**	**Thomson's gazelle** **(Gazella thomsoni)**
Alphaherpesvirinae *Varicellovirus*	Equine herpesvirus 1	Asinine herpesvirus 3 *Equid herpesvirus 8*	*Gazelle herpesvirus 1* *Equid herpesvirus 9*
Viscerotropic subgroup	Equine herpesvirus 4		
Dermatotropic subgroup	Equine herpesvirus 3	Asinine herpesvirus 1 *Equid herpesvirus 6**	
Gammaherpesvirinae *Percavirus*	Equine herpesvirus 2		
	Equine herpesvirus 5	Asinine herpesvirus 2 *Equid herpesvirus 7***	

Taxonomy of Equine Herpesviruses (EHV) adapted from Davison et al. (2009)[8] and Allen et al (2004).[9] The nomenclature assigned by the Herpesvirus Study Group of the International Committee on Taxonomy and Nomenclature of Viruses (ICTV) is added in italic.[10,11]

* Tentative species in the genus ** Unassigned species in the subfamily

At least five of the equine herpesviruses shown in Table 1 are described in the literature as equine pathogens; three of the subfamiliy *Alphaherpesvirinae*, namely *Equid herpesvirus 1, 3, 4* (EHV-1,3,4)[6] and two of the subfamily *Gammaherpesvirinae, namely Equid herpesvirus 2 and 5* (EHV-2,5).[12] Epidemic outbreaks can occur with equine alpha herpesviruses; EHV-1 is responsible for abortion, encephalomyelitis,[13,14] and respiratory disease outbreaks,[6,15] EHV-3 causes coital exanthema outbreaks,[16] and EHV-4 causes respiratory disease outbreaks.[17,18] All equine Gamma herpesviruses (EHV-2, EHV-5, EHV-7) cause mild respiratory disease in young animals and may have a role in the pathogenesis of Equine multinodular pulmonary fibrosis (EMPF), a rare interstitial lung disease affecting horses at all ages.[19,20]

1.1.2 Equine Alpha herpesviruses

The equid alpha herpesviruses 1 and 4 (EHV-1, EHV-4) cause worldwide epidemic outbreaks in horses, and have great economic impact on the equine breeding and competition industry.[8,21-37] The clinical presentations of EHV-1 infections are respiratory disease, abortion, neonatal foal death and myeloencephalopathy.[14,38-42] Large abortion outbreaks on breeding farms, called abortion storms,[42-51] as well as outbreaks of Equine Herpes Myeloencephalopathy (EHM)[39,41,42,52-56] have been reported worldwide. In contrast, EHV-4 infection mostly remains restricted to the upper respiratory tract and causes a mild upper respiratory tract infection that has high morbidity but low mortality. Rarely, abortion due to EHV-4 has been reported.[25,29,32,33,37]

Typical characteristics of alpha herpesviruses are their efficient and relatively short replication cycle as well as latency in sensory neurons, trigeminal ganglion or lymphocytes.[2,57] The predominantly intra-axonal transmission is reported to be the most efficient transmission pathway of all transmission routes. Release of virus from infected cells leads to replication and causes cytopathic cell injury, resulting in development of intranuclear eosinophilic inclusion bodies.[58] *In vitro*, alpha herpesviruses are able to infect many different cell types of various species. *In vivo* infection with alpha herpesviruses can cause infection in various host species however adaption of a virus to a single species is common.[58] Situations linked to stress, such as transportation, parturition, weaning and others, as well as administration of high doses of corticosteroids might enhance activation and spreading of the virus.[59,60]

1.1.2.1 Virion

A typical EHV-1 particle consists of approximately 30 discrete kinds of polypeptides.[61-63] Four morphologically distinct components build up the herpesvirus virion; the inner nucleoprotein core containing a linear double-stranded genomic desoxyribonucleic acid (DNA), the icosahedral capsid, the tegument and the envelope (Fig. 1).[64] The size of the virion itself is approximately 120nm, combined with the tegument and the envelope the size of the virus particle increases up to 300 nm.[65]

Figure 1: The herpesvirus virion

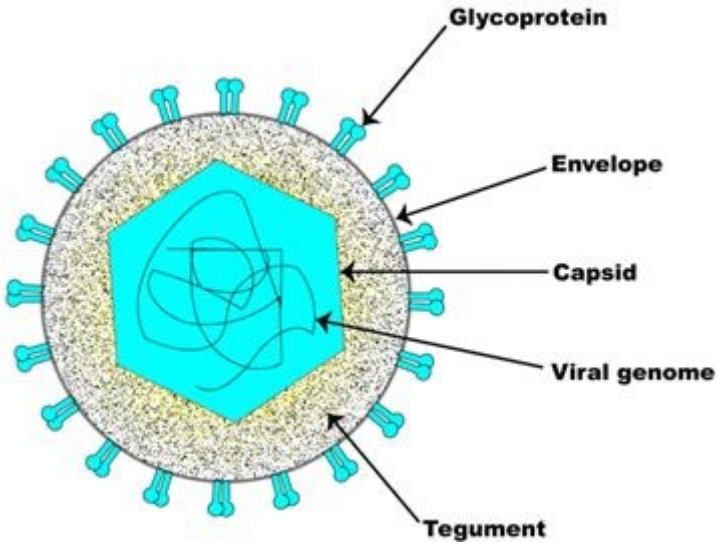


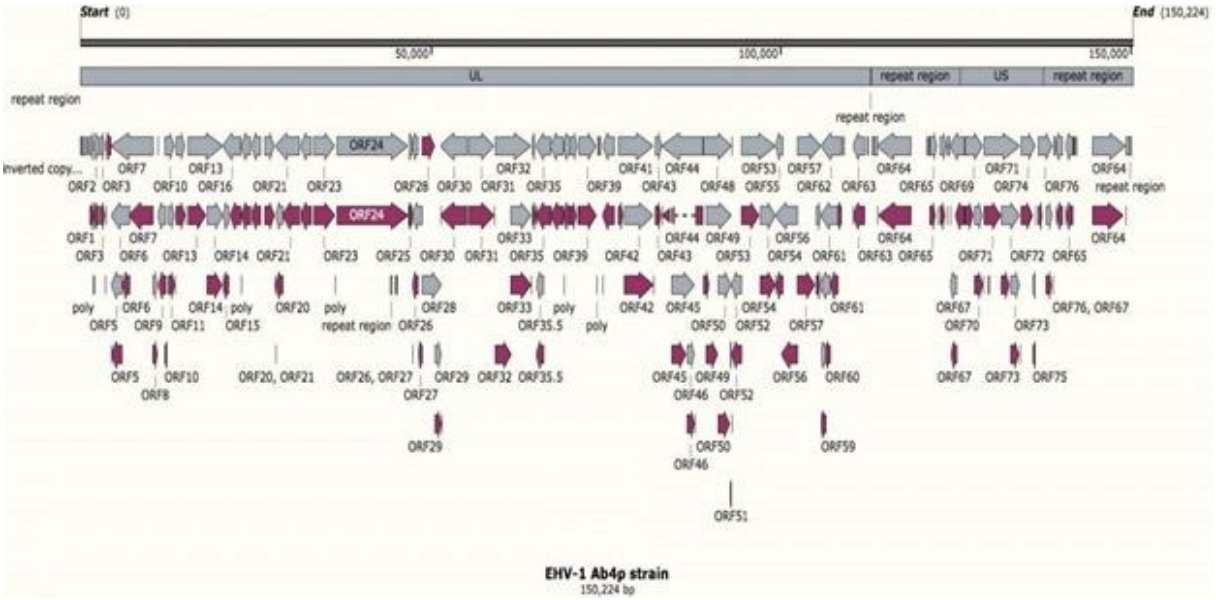
Illustration of the structural components of the herpesvirus virion by Oladunni et al. (2019)[66]

The capsid protein structure and arrangement of every herpesvirus is similar, involving 162 capsomeres.[67] The nucleocapsid contains a ring structure of 12 proteins that allows for the viral DNA to enter the capsid.[68] The amorphous tegument layer describes the area between the nucleocapsid and the envelope and consists of about 12 proteins which are involved in early infection stages and viral replication.[67] The envelope surrounds the nucleocapsid and the tegument and originates from patches of altered host-derived cell membrane.[69] Herpesviruses obtain their final envelope in the cytoplasm after passing the nuclear membrane. A primary envelope is acquired by budding of nucleocapsids at the inner leaflet of the nuclear membrane and fusion at the outer leaflet afterwards.[70] The eleven embedded glycoproteins (gp) are preserved across all alpha herpesviruses and therefore named according to the HSV-1 nomenclature.[67] The envelope gp are relevant components in entering a susceptible host cell, determination of host range, virus cell-to-cell spread, pathogenicity and immunologic response to infection. EHV-1 encodes an additional glycoprotein 2 (gp2) with homologues proteins only in EHV-4 and AHV-3.[67] Phylogenetic analysis using the glycoprotein G (gG) nucleotid sequences has suggested that AHV-3 is evolutionary much closer related to EHV-1 than either virus is to EHV-4.[71]

The full genome sequence of EHV-1 has been published in 1992 and 1998 by Telford et al., providing information about the genomic organization of the virus.[15,17] The size of the linear dsDNA genome of the EHV-1 virus is 150.2 kbp, containing 57% guanine +cytosine.[65] The genome involves 80 open reading frames (ORFs) encoding 76 different genes.[15] Generally, the arrangement of the EHV-1 genome is similar to other sequenced herpesviruses except for five genes (ORF1, 2, 67, 71 and 75) which are only encoded by EHV-1 with no structural homolog detectable in other sequenced herpesviruses.[9] The function of some of these genes remains unknown but it is supposed that they influence the unique adaption of the EHV-1

virus to the horse as their natural host.[9] Exemplary, the genomic map of the complete DNA-sequence of a pathogenic British isolate (strain Ab4) of EHV-1 is pictured by Ata et al. in 2018 using Snap Gene software program (Fig. 2).[72]

Figure 2: EHV-1 genome map



Genomic map of a EHV-1 Ab4p strain by Ata et al. (2018)[72]

A single non-synonymous nucleotid (nt) substitution of guanine (G) for adenine (A) at position 2254 in the viral DNA polymerase gene, encoded by ORF 30, results in a change of asparagine to aspartic acid at amino acid position 752 in the Ab4 strain. This genetic mutation in this strain is associated with occurrence of EHM,[73] whereas the genetically related non-neuropathogenic V592 strain is considered less virulent. In a survey, five Welsh Pony mares and one foal were challenged intranasally or by aerosol with the V592 isolate and monitored clinically and virologically.[74] All animals showed exclusively upper respiratory signs. The low virulence of this strain compared to highly virulent Ab4 and Army 183 isolates was associated with lower degree of endotheliotropism, which might be influenced by host immunity.[74] The Ab4 strain is also associated with more frequent abortion in mares.[75]

The genomic architecture of EHV-4 and EHV-1 are similar and a high degree of sequence identity (75-90% for most proteins) is reported.[71,76] The exception to this are the gG homologues, which only show an amino acid identity of 58% but are the only known EHV-1 and EHV-4 gp to possess strong, type-specific epitopes which can be used for differentiating EHV-1 and EHV-4 in diagnostic tests.[15,77]

1.1.2.2 Epidemiology

EHV-1 was initially described in 1933 at the Kentucky agriculture experimental station by Dimock and Edwards, causing epizootic abortion in mares.[78] Almost twenty years later, a large abortion outbreak in Spain was recorded, affecting nearly half of a group of 125 mares.[79] The abortion virus was isolated for the first time 16 years later in Western Germany from an aborted fetus and two foals that died shortly after birth.[80] The association between EHV-1 respiratory infections and abortion was first recognized in Europe in 1999.[81] Since then EHV-1 outbreaks have been described worldwide.

Due to the close relationship of EHV-1 and EHV-4, they were considered to be the same virus or minor variants of a single virus called EHV-1 for a long time. Since 1981, repeated investigations have shown distinct differences in the restriction endonuclease fingerprints of these two viruses. In the early 1990s, detection of a type-specific antibody response to EHV-4 gG, enabled differentiation of antibodies present in polyclonal sera. This lack of differentiation of the two viruses in early studies has to be considered when interpreting results of equine alpha herpesvirus studies of the past.[77]

Horses are exposed to EHV-1 and EHV-4 very early in life. Epidemiological evidence suggest that neonates are exposed to the virus shed by their dams and most of them seroconvert by six months of age, even when raised in a vaccinated population.[82,83] This is supported by studies from Australia and New Zealand that show that seroprevalence increases with age.[84,85]

1.1.2.3 Seroprevalence

Serological surveys conducted within defined populations, confirm a worldwide distribution of both viruses.[27,30,32,33,83,86-91] The worldwide prevalence ranges from 1 - 90% for EHV-1 and 26 - 100% for EHV-4. Seroprevalence based on region and age (older than 2 years) for both EHV-1 and EHV-4 is shown in Table 2.

Table 2: Seroprevalence of EHV-1 and -4 based on age and country

Country/Continent	EHV-1	EHV-4
Australia	26 - 30%[85,86,92,93]	99 - 100% [86,92-94]
Germany	82%[91]	95%[91]
Israel	1%[95]	99%[95]
New Zealand	21 - 70%[30,84,89]	90 - 100%[30,89]
Spain	51%[27]	26%[27]
Turkey	52%*[32]	77%*[32]
USA	80 - 90%[96-98]	80 - 90%[96]

*examined population includes horses and donkeys

Based on these reports EHV-1 appears to have significantly lower seroprevalence than EHV-4. There are however several factors that complicate interpretation of serological surveys of EHV-1 and -4, including a lack of availability of type-specific antibodies in the early 1990s,[92] duration of antibody response, frequency of reactivation of the virus, assay characteristics, host factors, season and vaccination status.

Test characteristics play major role as shown in a study where EHV-4 antibodies were overestimated compared to EHV-1 antibodies in a group of experimentally infected ponies using the Svanovir ELISA (Svanovir EHV1/EHV4-Ab ELISA, Svanova Biotech AB, Sweden).[27] Interpretation of data of seroprevalence surveys using conventional antibody tests such as virus neutralization and complement fixation are inaccurate.[99] Therefore, EHV-4 specific antibodies rather than EHV-1 immune response could have been measured in some older studies.[100] Peptide specific Enzyme Linked Immunosorbent Assays (ELISA) are currently used to detect specific antibodies against EHV-1 and -4.[92]

Results from EHV-1 and -4 seroprevalence surveys also have to be interpreted with regards to recent vaccination and natural infection.[101] After natural EHV-1 infection, the immune response conferring protection to reinfection lasts only a few weeks or months. Overall, duration of EHV-1 immunity after infection is short, and reinfection may occur after 3 - 6 months.[102,103] No comparable investigations on duration of antibody response for EHV-4 are available. EHV-4 may also be more endemic in equine populations compared to EHV-1 due to more frequent reactivation of latent EHV-4 infection without coincident disease, as is described for herpes simplex virus in humans.[104] EHV-4 infection occurs during the whole year and causes respiratory disease in a wide range of horses, while EHV-1 infection mainly occurs in winter causing abortion among mares in the late stage of gestation.[105] Hence, season of sampling may influence seroprevalence data.[105]

1.1.2.4 Antigen (virus) prevalence

Prevalence of antigen (virus) detection in healthy horses by PCR out of nasal swabs ranges from 0 - 33% for EHV-1[26] and 0 - 9% for EHV-4.[26,106] Virus detection in lymphoid tissue from healthy horses ranges from 10 - 54% for EHV-1[30,34] and 6 - 83% for EHV-4.[24,34] In animals with respiratory disease, PCR positive results from nasal swabs range from 3 - 7% for EHV-1[28] and 6 - 67% for EHV-4.[33,106] EHV-1 and -4 antigen prevalence in nasal swabs from healthy horses and horses with respiratory disease in various countries are shown in Table 3 and 4 respectively. Prevalence of neuropathogenic strains detected in lymphoid tissue, various organs of aborted fetuses and horses with EHM are also presented in Table 3 and Table 4. Age of the sampled population, vaccination history and differentiation of abortion and EHM outbreaks are not shown in these data.

The neuropathogenic strain of EHV-1 (D_{752}) has a worldwide distribution.[22,24,30,36,44-46,73,107-109] The estimated prevalence detected in lymphoid tissue from healthy horses ranges from 6 - 18%.[24,30,109] In confirmed EHV-1 outbreaks (EHM and abortion) the median percentage of neuropathogenic strains is 9%, but the range is wide with 0 - 90%, depending on region and study.[22,24,30,36,44-46,73,107-109]

Table 3: Prevalence of EHV-1 and -4 nasal shedding in healthy horses based on PCR

Country/Continent	**EHV-1**	**EHV-1 D_{752}**	**EHV-4**
Australia			9%[110]
Ethiopia	0%[26]		0%[26]
New Zealand	33%[30]	6%[30]	
Uruguay		92%[24]	
USA		17 - 18%[34,109]	

Table 4: Prevalence of EHV-1 and -4 nasal shedding in horses with clinical signs compatible with EHV-1 infection based on PCR

Country/Continent	EHV-1	EHV-1 D_{752}*	EHV-4
Argentina		7%[111]	
Australia			6%[110]
Brazil		0%[55]	
Ethiopia	7%[26]		8%[26]
France		24%[112]	
Germany		11%[113]	
India		4%[44]	
Ireland		13%[108]	
Italy		90%[22]	
Japan		3%[36]	
Poland		0 - 3%[43,46]	67%[33]
Turkey		0%[48]	
UK		27%[107]	
USA	3%[28]	11 – 19%[114,115]	

*EHV-1 D_{752} detected in lymphoid tissue, different organs of aborted fetus and horses with EHM

1.2.2.5 Latency

Latency describes the non-replicative, non-immunogenic stage which allows persistence of the virus in the body. Hallmark of latency is the restriction of viral gene expression which results in failure to synthesize certain viral proteins and hence absence of infectious virus particles.[38] EHV infection can become latent in approximately 80% of horses,[116] either in lymphocytes (both circulating and those in draining lymph nodes) or sensory nerve cell bodies.[116-118] The trigeminal ganglia are the preferred primary site of EHV-1 latency.[119,120] Latency occurs predominantly in CD5+/CD8+ T-lymphocytes: in 80% of these cells, reactivation of EHV-1 could be detected in venous blood by indirect immunofluorescence whereas CD5+/CD-8-/CD4- cells are less frequently involved in EHV-1 latency (20%).[121] In EHV-1 infected horses, the expression of the EHV-1 genome is suppressed and only the latency-associated transcripts (LATs) are present in infected cells.[67,118,120]

Reactivation of virus in a latently infected animal can serve as a source of infection for other horses and may or may not be accompanied by clinical signs.[59,122] EHM or abortion cases in a closed group of horses have occurred without a known external source of EHV-1 infection.[123] Similar epidemiological observations were made on outbreaks at stud farms in Australia and

in the UK.[124] Both reports conclude that reactivation of a latent infection in one of the mares which had been present on the stud for some months was the source of the outbreak.[125,126] Triggers for EHV-1 reactivation are stressful conditions such as transport, sales, competitions, pregnancy and parturition[127,128] or experimentally administration of high doses of glucocorticosteroids.[59]

The local reactivation of the virus in the blood vessels of the pregnant uterus can proceed with or without concurrent lytic respiratory infection and therefore with or without shedding the virus via nasal secretion.[127] The initial respiratory infection that lead to latency of the virus likely occurred months or years before the abortion or EHM.[96]

Coinfection with the neuropathogenic EHV-1 D_{752} and non-neuropathogenic EHV-1 N_{752} strain is a common observation.[109] Infection with both variants of the virus was shown in a case were the mare was shedding a neuropathogenic variant, whilst the aborted fetus was positive for a non-neuropathogenic variant.[54] On the other hand, latency of the original infective strain in submandibular lymph nodes (SLN) in 24 weanlings five years after experimental infection was shown.[129]

Co-infection with other herpesviruses also plays a role in re-activation of latent infections. EHV-2 may act as a transactivator for the IE gene promotor on a latent EHV-1 virus.[130] Another study showed that experimental EHV-1 infection was able to reactivate a latent EHV-4 infection.[131] A recent study from Ireland investigated molecular characterization of EHV-1 strains in several outbreaks on selected premises over multiple years.[108] Identical molecular virus profiles were present in the horses from the same farm within a year, whereas virus strains were rarely identical on the same premises in different years. These results suggest reintroduction of a new EHV-1 virus strain rather than reactivation or persistent circulation of a latent infection.[108]

1.1.2.6 Transmission

EHV-1 is a highly contagious virus and is usually transmitted by direct contact with nasal discharge of an infected animal or object. Contaminated feed, water, equipment or aerosols are less common routes of transmission.[14,18] Large amounts of infectious particles are present in fetal and placental tissue from EHV-1 abortions and can be transferred via direct contact (e.g. paddock mates) or via fomites (e.g. shoes, clothing of people).[9,132] Outbreaks of viral respiratory disease are commonly reported in two- and three-year-old horses in training, on racing circuit and in race- or show barns, in which horses from different origins are kept together in enclosed spaces.[96]

Investigations of transmission of EHV-1 and -4 in stud farms suggested that both mares and foals can act as a pool for virus transmission.[86,133] Seroprevalence on a large Thoroughbred stud farm revealed more than 99% of mares and foals positive for EHV-4 while only 26% of

mares and 11% of foals were tested positive for EHV-1 antibodies.[86] Based on the results of mare-foal pairs the authors concluded that EHV-1 infection occurred in foals prior to weaning at less than five months of age. EHV-1 infection in foals was active and not a residual of maternal derivation as the half-life of maternal EHV-1 antibodies in foals' serum is 31 days whereas the average age of the foals was 124 days. EHV-1 antibody positive foals were four times more likely to have an EHV-1 antibody positive dam compared to seronegative foals. Presumably, mares were a source of EHV-1, from which their foals became infected in these cases. Foals with seronegative dams were likely infected due to close contact with other infected foals.[86] Results of this study suggest that transmission of the virus was a consequence of reactivation of a latent EHV-1 infection in a sub-group of the mares, possibly, due to stress of parturition, estrous activity or lactation. Close contact of the mares with their foals as well as amongst foals is a plausible reason for transmission of the virus.[86] Epidemiologically, a cyclic but mostly quiet pattern with mares serving as a continuous source of infectious virus particles for the foals during the breeding season is suggested. Dams undergo recrudescence of latent viral infection in stressful situations like pregnancy/parturition and horizontally transmit the virus to the foal.[38,67]

Whether venereal horizontal transmission by infectious semen or an effect on stallion fertility can occur, is unclear. In one study, 13% of 390 semen samples were EHV-1 positive but there was no effect on stallion fertility.[134] Another study showed shedding of EHV-1 in semen after natural infection up to 20 days after onset of fever, but virus isolation from semen was not successful.[135]

There is little information about the duration of EHV-1 nasal shedding in horses with EHM. The conventional belief that horses are no longer shedding infectious virus particles when showing signs of EHM, should be abandoned. A large outbreak demonstrated the threat of nosocomial EHV-1 infection associated with hospitalization of horses with EHM without biosecurity precautions. Some horses were still shedding the virus in nasal secretions at the time they were hospitalized for EHM and were able to infect other hospitalized horses.[136] A more recent study showed EHV-1 viral shedding up to nine days after the onset of EHM due to natural EHV-1 infection.[54] The severity of clinical signs can also not reliably predict duration of EHV-1 shedding.[54] Hence, it is currently recommended that biosecurity measures should be implemented for a minimum of 14 days beyond onset of clinical signs of EHM.

Negative effects of EHV-1 on the equine industry include interruption of training, especially in young athletes due to pyrexia and respiratory signs, and impaired growth of the horse population due abortion. Neurologic outbreaks are also a severe threat to the horse industry. Deaths of horses, disruption of breeding or training schedules, cancelation of horse events and extensive movement restriction with consequently management difficulties at racetracks and training facilities may occur.[14]

1.1.2.7 Pathogenesis of EHV-1 and -4 in general

Initially, infection of the epithelial cells of the nasal mucosa or nasopharynx occurs.[137] The virus can use different pathways for entering the cell, either directly fuse with the plasma membrane or endocytosis followed by fusion with an endosomal membrane.[138] The virus uses different receptors to enter the cell,[139] including equine major histocompatibility complex 1 (MHC-1) and cellular integrins.[138,140] Once the virus is inside the cell, the lytic replicating cycle is started, leading to necrosis and inflammatory cellular response. Distinct herpetic lesions of mucosal membranes and shedding of infectious particles ensues.[101] After infection via the respiratory tract, the virus moves into the cells and hence is not accessible for neutralizing antibodies or other components of the immune response.[141]

The predisposition to other secondary bacterial infections due to the local damage of the respiratory epithelium has been demonstrated in a survey of respiratory viruses in New Zealand horses, in which multiple respiratory pathogens from horses with clinical signs of respiratory disease were detected.[142]

Invasion of the deeper connective tissue occurs by hijacking migrating mononuclear cells.[143] Consequently, EHV-1 can invade the reticuloendothelial system and the lymphatics to infect circulating leucocytes and endothelial cells of blood vessels.[101,137] Reaching the respiratory tract-associated lymphoid organs, a second round of replication in leukocytes starts, leading to a state of cell-associated viremia by spreading the virus via lymph and blood-vascular circulation.[144,145] Viremia enables further transportation to tertiary replication sites, such as the vasculature of the pregnant uterus or the Central Nervous System (CNS).[143]

Pathogenic lesions occur due to vasculitis, thrombosis and ischemic damage.[146] A strong, cell-associated viremia is crucial to the pathogenesis of EHV-1 and is supposed to be the determining difference in the pathogenicity of the higher and lower virulent EHV-1 strains as well as the closely related EHV-4.[143,147,148] The latter replicates mainly in the upper respiratory tract and clinical signs other than respiratory disease are uncommon.[143,149] An experimental trial using nasal mucosal explants as an infection model showed considerable spreading of the EHV-1 from the epithelium to the connective through the basement membrane where mononuclear leukocytes were infected, while EHV-4 infected leucocytes were extremely rare.[148]

Main clinical signs in horses with EHV-1 and -4 infection are a biphasic fever and mandibular lymphadenopathy. The first fever peak is measured 36-48 hours following nasopharyngeal instillation of a viral inoculum. Secondary fever occurs between days 5 and 10, when the virus is replicating in the peripheral blood mono-nuclear cells. This febrile phase is associated with high shedding of either EHV-1 or EHV-4 virus.[150] Generally, severity of disease depends on age, physical condition of the host, type of infection (primary infection/reinfection or reactivation of latent virus), immune status of the host and pathogenic potential of the virus strain.[73]

1.1.2.8 Respiratory Disease

The first lytic replication cycle takes place in the respiratory epithelium of the upper airways. As early as 12 hours post infection, first progeny virus and viral antigen are detectable in the respiratory epithelium of an infected horse and spreading to the respiratory endothelium may occur within 24 hours.[67,151] Incubation time depends on pathogenicity of the virus strain and ranges from 1-3 days[152-154] up to 10 days.[5]

Clinical signs resemble those of other equine viral respiratory pathogens (e.g. influenza virus, rhino- or adenovirus and equine arteritis virus) and include primary rhino-pharyngitis and tracheobronchitis.[96] Although most of infections proceed without clinical signs, some naïvely exposed young horses may show coughing and nasal discharge.[9,96] Risk factors for EHV-1 and -4 respiratory outbreaks include overcrowding, heavy parasite burden, poor nutritional state, climatic extremes, additional underlying disease and the mingling of animals from different social groups.[96] After experimental infection using the virulent Ab4 strain of EHV-1, fever in a biphasic pattern up to 10 days was observed.[152-154] Lymphadenopathy, affecting especially submandibular and occasionally retropharyngeal lymph nodes, mucoid and mucopurulent nasal discharge, depending on secondary bacterial infection, conjunctivitis and serous ocular discharge, accompanied by moderate depression and anorexia, are reported.[9] On hematologic analysis leucopenia (lymphopenia and neutropenia) may be present and in foals, bronchopneumonia is reported in severe cases.[155-157]

Overall, the course of the disease proceeds acutely with nasal shedding over the first few days after infection and prognosis is good with spontaneous recovery after two weeks of onset of infection in most of the cases. If secondary bacterial infections occur, recovery is prolonged and prognosis undermined.[96] Non-specific bronchial hypersensitivity after recovery of an EHV-1 infection is an additional reported sequelae which may lead to poor performance syndrome with clinical signs similar to obstructive pulmonary disease.[158]

1.1.2.9 Abortion, Neonatal and Perinatal Disease

EHV-1 reaches the reproductive tract through cell-associated viremia.[159] The virus first causes vasculitis in the small vascular network of the glandular endothelium of the microcotyledons followed by widespread vasculitis within 9 to 13 days after infection.[9,160-162] Microthrombosis within blood vessels may support thrombo-ischemic necrosis of the cotyledons and intercotyledonary stroma and causing detachment of the fetus from the placenta.[162] The fetus dies due to anoxia and can be aborted even before any detectable concentrations of virus is transferred via the placenta to the fetus.[161] Severity of disease depends on different factors such as virulence of the EHV-1 strain, magnitude and duration of viremia and the hormonal state of the mare.[9,67,75] The magnitude of viremia is more important than the duration of viremia.[75] Hormones like prostaglandin and chorionic gonadotropin (CG) are reported to reactivate the virus and initiate abortion.[9,67]

Due to premature detachment of the fetus from the placenta, stillbirth or birth of weak neonatal foals occurs.[163] Mares infected with the virus, may abort spontaneously without prior signs of respiratory disease.[75,161,164,165] The infected foals show multi-organ pathologies and shows clinical signs at birth or within 1-2 days of birth.[122,166,167] Prognosis is grave and there is only supportive therapy with little success for stopping continuous deterioration due to severe respiratory distress, diarrhea as well as neurological deficiencies, manifesting in visual and vestibular defects.[155,159,166] It is proposed, that congenital EHV-1 infection can be epizootic and may occur in association with an outbreak of EHV-1 induced abortion.[96] Rarely, congenital infection with EHV-4 can cause neonatal foal disease.[96]

1.1.2.10 Myeloencephalopathy

Similar to the spread to the uterus, the cell-associated viremia is also fundamental for invasion and efficient spreading to the CNS.[102] Due to its intracellular location and exclusively cell to cell transmission, the virus remains undetected by the immune system, especially the important disease preventing neutralizing antibodies.[99,102] Vasculitis in the CNS is a result of direct damage of the endothelium during EHV-1 replication or immune complex formation (Arthus-type reaction).[18] Vasculitis, with or without hemorrhage, edema, microthrombosis leading to thrombo-ischemic necrosis are the pathological sequelae.[9]

Equine Herpes Myeloencephalopathy (EHM) may occur in horses with our without previous signs of upper respiratory tract disease or abortion.[168] Clinical signs depend on severity and location of the pathological damage. Neuropathogenic strains may lead to more severe clinical signs but approximately 14 – 24% of EHV-1 strains from horses with EHM do not show the genetic mutation.[73,148] There is no satisfactory explanation for variable incidence of EHM and different clinical manifestations observed during outbreaks.[122,169] Clinical signs vary and occur in the first week after infection.[9,155] Neurological deficits, including temporary ataxia to complete paralysis and especially affecting the hindlimbs, urinary and fecal passage problems are reported with the peak between day 2 and 3 after onset of clinical signs.[9,122,170] Prognosis

for non-recumbent horses is good whereas recumbent horses are generally euthanized due to further sequelae such as pneumonia, colic or bladder rupture.[9,122]

An increased likelihood of EHM development in association with more frequent vaccination against EHV-1 has been suggested.[18,146] This has to interpreted with caution, as it is difficult to assess the impact of vaccination alone, isolated from other factors.[14] Many factors like age of the horse, prior exposure to the virus, individual response to the vaccine and carriage of both EHV-1 genotypes in latent infected horses may also influence the development of EHM.[14,52,149]

1.1.2.11 Occular Disease

In some infections with highly virulent EHV-1 strains, foals may show ocular lesions such as chorioretinitis and uveitis within 3-5 weeks of upper respiratory tract disease.[171] After experimental infection with EHV-1, 50 - 90% of horses developed chorioretinal lesions.[172] Young foals in contact with EHM-infected horses have an increased risk of developing uveitis.[122]

1.1.2.12 Diagnosis of EHV-1 and -4

As a single antibody titer alone is not a confirmation of clinical disease, antigen detection is necessary for diagnosis of clinical cases. A quick diagnostic technique is preferable for clinical cases as a fast diagnosis needs to be achieved to minimize the spread of disease within a population of horses. PCR is a useful diagnostic tool for detection of genomic material of EHV-1 and can be performed on various sample material such as nasal swabs/nasal discharge, aborted fetus, placenta, brain and spinal cord, paraffin-embedded archival tissues and infected cell cultures.[173-177] By using novel PCR- platforms such as real time PCR (RT-PCR), quantification of viral load can be assessed.[178] Nucleic acid from a viable and non-viable virus can, however, not be distinguished with this method. Agreement between positive PCR results and virus isolation is reported between 85 and 90%.[9] Particularly in samples with low levels of DNA copies, correlation with compatible clinical signs is necessary to confirm a diagnosis. RT- PCR is also able to distinguish between the neuropathogenic and non-neuropathogenic strain.[179,180] Immunofluorescence staining applied on impression smears from nasopharyngeal swab is another fast detection technique for detecting viral EHV-1 antigen.[9,96]

Cell culture followed by virus isolation remains the gold standard but is only recommended when investigating EHV-1 outbreaks or for research purposes. For successful virus isolation it is necessary to take samples at early stages of the disease. The peak of virus shedding may already have passed at the onset of neurological signs and isolation may be impaired by interfering local antibodies.[124] The cytopathic effect (CPE) appears quickly in cell culture and

remains unique for herpes viruses, showing clusters of rapidly enlarging, rounded, and detached cells.[9]

Serologic analyses including ELISA and Virus Neutralization Test (VNT) are recommended by OIE for confirmation of EHV-1 clinical cases and infection prevalence surveillance.[181] A peptide-based ELISA is a simple, very specific, rapid, sensitive and relatively cheap serological diagnostic tool that is able to distinguish between EHV-1 and EHV-4.[91] Considering the course of neutralizing antibodies, they rise quickly after natural infection and between 5 - 8 days after experimental infection.[84] Moreover, they decline rapidly after natural infection as well in vaccinated animals. A four-fold increase in paired serology titers taken 10-14 days apart provides evidence of acute infection. In neurological cases a diagnosis based on antibody rise can be difficult as EHM occurs later in the disease and the antibody rise might already have occurred.[80,146] The antibody concentration can also help in establishing a diagnosis. High antibody titers indicate natural infection rather than residual of vaccination titers.[146]

1.1.2.13 Immunity against EHV

In the early stage of experimental infection with a virulent EHV-1 strain, components of the virus are detected within 12 hours post infection (hpi) in regional lymphoid tissue.[182,183] This suggests close interaction of the virus with the immune system of the host already in the early stage of infection. Several in vitro studies showed fast upregulation of pro-inflammatory cytokines for example in a endothelial cell model with either neurovirulent or non-neurovirulent EHV-1 strains 10 hpi[184] as well as type -I IFN induction 3 hpi.[185-188] Inflammatory cytokines activate the adaptive immune system for elimination of viral antigen, but induction of pathologies may occur due to activation of coagulative responses.[66]

Three types of immune responses are required for protection against EHV-1, including combat of free infectious virus particles by both mucosal and systemic virus neutralizing (VN) antibodies as well as cytotoxic T lymphocytes (CTL) to lyse virus infected cells.[159] Response of the humoral immune system by production of specific antibodies against the virus remains generally only temporary.[189,190] Overall, duration of EHV-1 immunity after infection is short and reinfection may occur after 3 - 6 months.[102,103] Nevertheless mares with EHV-1 abortion normally do not abort in the following year, suggesting that the immune response to EHV-1 infection is complex and probably lasts longer.[191] There are no reports in the literature of a horse being affected by EHM twice within two consecutive years or even within a longer time frame. This however has to be interpreted with caution as mortality for EHM is high, the disease is rare and horses might be lost to follow up.
Interestingly, the maternal serum VN antibodies as well as IgG levels decline fast in newborn foals and are absent in the third month of life. These antibodies kinetics agree with the previous reported half-life of 31 days.[192] Therefore determination of the best time frame for immunization of foals is important but also difficult, as early infection with EHV-1 and 4 has been documented in several cases.[82,133]

Virus neutralizing antibodies provide protection up to one year but complement-fixing (CF) antibodies only last for three months and show cross reactivity with EHV-4. Both antibody types are produced within two weeks after infection.[190] The humoral immune system generally targets the epitopes of the surface of envelope gp of EHV-1 virus and all of these antibody isotypes have been detected in EHV-1 infected horses.[67,76,193-195] Especially mucosal IgA VN antibodies promote important local protection in the upper respiratory tract (URT). Reduction in nasopharyngeal virus shedding following intranasal challenge infections of ponies 3 and 13 weeks after first infection was observed.[196] But once cell-associated viremia is established, humoral immunity is inefficient. Therefore, high EHV-1 antibody titers provide information about exposure to the virus in the past but do not seem to correlate with protection against disease, such as EHM and abortion. There are case reports of clinical EHV-1 infection with a high titer of neutralizing antibodies.[52,66]

Elimination of intracellular pathogens requires activation of CTL. A study showed upregulation of CTL response after experimental EHV-1 infection with IFN-γ playing a central role in activation of antigen-presenting cells and enhancement of the antiviral effects of circulating cytotoxic CD8 T - cells.[197] Investigation into correlation of level of CTL and clinical signs displayed higher concentrations of CTL associated with fewer clinical signs in adult ponies after history of EHV-1 infection compared to young ponies with low EHV-1 specific circulation CTL.[198] Quantification of CTL or memory cells may be useful for estimating efficiency of EHV-1 vaccines in horses.

1.1.2.14 Vaccination

Development of an effective vaccine against herpesvirus infection remains difficult due to induction of latency and other strategies of the virus to avoid components of the host innate and adaptive immune system.[189] Therefore, vaccines have to invoke strong and sustained levels of humoral and cell-mediated immunity against the virus as well as block the development of cell-associated viremia.[66] Modified live vaccine and inactivated whole virus vaccine are currently available (Tab. 5), however only one vaccine contains both EHV-1 and EHV-4.

Table 5: Current commercially available EHV-1 and EHV-4 vaccines

Vaccine	Type	Indication and effect	Vaccination scheme*	Content
Duvaxyn® EHV-1,4, (Zoetis, Pfizer)[192,199]	IWVV	- Prevent abortion caused by EHV infection in immunocompetent mares - Decrease respiratory signs and risk of abortion - Reduce virus shedding after infection with a field strain	- Foals • 1st dose: ≤ 6 months (m) • 2nd dose: 4 - 6 weeks (w) after 1st dose • Revaccination every 6 m - Pregnant mares: following AAEP guidelines[200] (https://aaep.org/guidelines/vaccination-guidelines)	438/77 EHV-1 405/76 EHV-4[199]
Pneumabort K®+1B (Zoetis)[201]	IWVV	- prevent abortion due to EHV-1 infections - Prevent respiratory infections caused by EHV-1p and EHV-1b[53,202-205]	- Foals • 1st dose: 6 m • 2nd dose: 3 – 4 w after 1st dose • 3rd dose: 6 m after the 2nd • Revaccination every 12 m - Pregnant mares: following AAEP guidelines[200]	1p EHV-1 1B EHV-1 [201,206]
Prevaccinol® (MSD)[192,207]	MLV	- reduce EHV-1 infection - reduce clinical signs of EHV-1 induced respiratory disease[53,101,208,209]	- Foals: 2-dose series: • 1st dose: ≥ 6m • 2nd dose: 3 - 4 m after 1st dose • Revaccination every 6 m - Pregnant mares: following AAEP guidelines	*Rac-H* EHV-1 strain[207,210] (at least 10^6, max. $10^{7,7}$) $TCID_{50}$ **
Rhinomune® (Boehringer Ingelheim)[53,209,211,212]	MLV	- aid in the prevention of respiratory disease caused by EHV-1[53,101,208,209]	Foals: 2-dose series: • 1st dose: ≥ 3 m • 2nd dose: 3 – 4 w after 1st dose • Revaccination every 3 m - Pregnant mares: at 2 m of gestation every 3 m	*Rac-H* EHV-1 strain[210]

* $TCID_{50}$: Tissue culture infectious dose 50%
AAEP : American Association of Equine Practitioners
IWVV: inactivated whole virus vaccine; MLV: modified live vaccine

Virus neutralizing antibody titers of at least 1 in 10^3 can be achieved by stimulation of the humoral immune system by integration of ISCOMs (immune stimulating complexes) in inactivated vaccines. A reduction in the amount and duration of virus nasopharyngeal shedding was achieved by vaccination using such techniques.[190] However, high pre-infection VN antibody titers may not be correlated with protection against abortion and duration of cell-associated viremia following challenge infection with EHV-1.[213]

A mucosal humoral immune response has been suggested to provide better protection compared to VN antibodies. A study analyzed the mucosal humoral immune response of weanling foals following experimental infection with virulent EHV-1 or vaccination with either an IWVV (Pneumabort K®) or MLV (Rhinomune®) according to different protocols.[196,214] Finally, all weanlings were challenged intranasally with virulent Army 183 (A-183). The control group underwent only challenge infection. Virus-specific IgA dominated the mucosal antibody response elicited in weanlings inoculated with A183, being detectable at up to 3.1 g/mg total IgA 13 weeks after challenge. However, neither inactivated EHV-1 administered intramuscular nor attenuated EHV-1 administered intranasally induced detectable mucosal antibodies.[196]

Investigations of the same vaccine often showed different results. This is likely due to differences in the immunological status of the study population, including animals with variable age and unknown history of prior viral exposure.[215] Contemporary vaccines irrespective of the type, MLV or IWVV, result in a partial protective immune responses, by stimulating high titers of circulating VN antibodies. However, most current vaccines are unlikely to stimulate cytotoxic effector lymphocytes. The important role of CTL activity in terms of protective immune responses has however been recorded in several studies.[198,216,217]

There are several experimental studies showing that both MLV[53,192,209,218] and IWVV[53,190,192,218-222] vaccines are safe to use and offer at least partial protection. Reduction in clinical disease such as abortion in mares and respiratory signs in adult horses and foals are reported for both MLV[53,223-225] and IWVV.[53,190,192,202,219,220,226] Efficient protection against disease outbreaks has not yet been demonstrated. Reduced viral shedding has been demonstrated in multiple investigations of MLV[53,192,209,218] and IWVV[53,190,218,219,221] but decrease in cell associated viremia is reported in MLV only in one study following a challenge trial in previously vaccinated horses with a live attenuated TS German abortion isolate (C-147).[192,227] Stronger influence on cell associated viremia was more frequent demonstrated for inactivated vaccines.[190,222,228]

A better efficacy of MLV vaccines compared to IWVV has been suggested, but data are conflicting. A study investigated reduction of EHV-1 viremia and nasal shedding by commercial vaccines: Three groups of 8 yearling ponies each were vaccinated with either an MLV (Rhinomune®, Boehringer Ingelheim Vetmedica, Inc.), an IWVV (Pneumabort-K®, Pfizer Animal Health) or a placebo in a blinded, randomized challenge trial.[53] Both vaccines reduced clinical disease and nasal viral shedding but only the IWVV group showed a reduced

number of days of viremia.[53] However, reduction of clinical signs was greater in the MLV group. These findings are supported by a similarly conducted challenge trial, comparing an IWVV (Fluvac Innovator 6 combination vaccine, Fort Dodge) and MLV (Rhinomune™, Pfizer) with a placebo.[209] Challenge infection was performed by intranasal delivery of aerosolized virus isolate obtained from a an EHM case. Neurological signs and fever were significantly lower in the MLV compared to the IWVV and control group. Virus shedding from the nasopharynx was almost undetectable in the MLV group and significantly lower when compared to the IWVV and control groups.[209] However, a large vaccination trial comparing efficacy of an IWVV (DuvaxynEHV1,4™, Pfizer) and a MLV (Prevaccinol™, Intervet, MSD) regarding serological response and protection against abortion did not show any difference between the two groups.[192] Magnitude and duration of humoral response assessed by serum neutralization assays and probing for EHV-1-specific IgG isotypes as well as incidence of abortion was not significantly different in this investigation.

More recently developed vaccines include an EHV-4 strain in addition to EHV-1. However, investigations of efficacy of these combined vaccines focus on EHV-1 with only little published data on the EHV-4 content of the vaccine. One study examined the severity of clinical disease, serum neutralizing (SN) and CF antibody response and viral shedding in a population of mares and foals vaccinated with an EHV-1,4 vaccine (Duvaxyn® EHV-1,4, Pfizer), followed by EHV-1 and -4 experimental infection.[219] The mares were vaccinated three times (5., 7., 9. month of gestation) and infected with an abortogenic Ab4 EHV-1 strain whereas the foals received two vaccinations in an interval of 4 weeks and were infected with both, a EHV-1 and EHV-4 field strain. Prevention of abortion in mares and respiratory disease in foals as well as a clearly reduced duration and amount of nasopharyngeal EHV-1 and -4 virus shedding were shown.[219] Serum neutralizing and complement fixing EHV-1 and -4 antibody titers were induced two weeks after first vaccination and were boosted after second vaccination, however they never reached the concentrations induced after virus challenge.[219] It is suggested, that antibody response is individually variable but generally shorter against EHV-1 compared to EHV-4.

In horses, most vaccines, including EHV-1,4, are administered intramuscular. Local intranasal vaccine delivery might be a more efficient way to induce local humoral immunity due to imitation of natural infection route.[215] During the decade of the 1940's, a hamster-adapted live virus vaccine was tested however accompanied by adverse side effects. Intranasal delivery of this vaccine to young horses protected them from challenging EHV-1 infection one month later. Further infection programs to immunize mares against virus abortion were conducted. During the next decade, the incidence of virus abortion was reduced from 15% in unvaccinated animals to 1% after vaccination.[229-231] However, safety problems concerning vaccine-induced abortion continued.[232]

More recently, a series of efficacy studies using an intranasal MLV containing a German abortion isolate (C-147) were conducted.[223,227,233] Prevention of abortion in pregnant mares[223] as well as efficacy against respiratory disease of adult horses[227] and reduction in clinical disease in foals was achieved.[233] No significant differences in viral shedding between

vaccinated and non-vaccinated mares or foals was shown. Vaccinated foals developed cell associated viremia as frequent as unvaccinated animals.[223,233] Only the yearlings displayed reduced viral shedding and cell associated viremia six weeks after challenge infection compared to the non-vaccinated animals.[227]An intranasal booster has also been suggested as an emergency strategy during an EHV-1 or EHV-4 outbreak to limit the spread of infection.[215] Overall results are encouraging but safe use of the vaccine has to be considered and has not yet been fully established.

Despite the fact that EHV vaccination cannot completely protect against infection, disease or spreading of the virus, overall, vaccination provides a substantial degree of protection in horses following exposure to EVH-1 and EHV-4. Widespread vaccination and improved management of breeding stock have been reported to reduce abortion storms in the USA by 75%.[5] Similar assumptions are made for Europe, due to reduced amount and duration of viral shedding in vaccinated animals.[215] In contrast, there are only few investigations regarding EHM following vaccination. Supporting published data in terms of efficient protection against EHM with any vaccine are missing.[52,234-236]

A strategy to protect young foals against EHV-1 is to increase amount of maternal antibodies in foals by vaccinating the pregnant mare against EHV-1, however the impact of maternal antibodies on EHV-1 is not completely understood.[192,237] Immunization of the foal is another strategy, but generally poor reactivity of the foal's immune system to primary vaccination is reported. Inactivated whole EHV-1,4 or sub-unit vaccinates administered to foals less than 5 months of age was not successful in mounting an immune response,[133,237] however vaccination of foals older than 5 months of age with a live-attenuated EHV-1 resulted in some degree of protection against signs of disease, reduced virus shedding and presence of cell-associated viremia after challenge infection with EHV-1.[233] The immature antigen presenting cell function for foals may contribute to the weak immune responses to vaccination. This has been shown in context with the absence of an immune response after vaccination with a DNA vaccine against *Rhodococcus equi*.[238] Pre-existing EHV-4 antibodies may also interfere with EHV-1 immunization.[133]

Determination of the best timeframe for immunization of foals include considerations about antibody kinetics and subclinical infections in the early age. Half-life of maternally derived VN antibodies is less than 4 weeks[192] and their absence in the third month of life contrasts with early EHV-1,4 infections at the age of 11 days.[93] It is currently not known, if these subclinical infections lead to cell associated viremia and establishment of latency. If this scenario holds true, prevention of latency by vaccination has a very short window of opportunity. Overall, the best timeframe for immunization of foals is difficult establish.

The ideal vaccination scheme and type of vaccine are under debate. Recommendations of the American Association of Equine Practitioners (AAEP) include vaccination of every horse at risk in the following scheme for adult horses (Tab. 6) and foals (Tab. 7).[239] The recommended

vaccination scheme of the Vetsuisse Faculty of Switzerland is similar to the reference of the AAEP and is presented in Table 8.

Table 6: Recommended EHV vaccination scheme for adult horses (AAEP, 2020)[200]

Broodmares	**Other adult horses (>1 year of age) Vaccinated**	**Other adult horses (>1 year of age) Unvaccinated**	**Comments**
3-dose series with product labeled for protection against EHV abortion Give at 5, 7, 9 months of gestation	Annual (see comments)	- 3-dose series: • 2nd dose 4-6 weeks after 1st dose • 3rd dose 4-6 weeks after 2nd dose	Consider 6-month revaccination interval for: 1) Horses less than 5 years of age 2) Horses on breeding farms in contact with pregnant mares 3) Performance or show horses at high risk

Table 7: Recommended EHV vaccination scheme for foals (AAEP, 2020)[240]

Foals (<12 months of age) of mares vaccinated in the prepartum period against EHV abortion as well as for foals of unvaccinated mares
Inactivated or modified live vaccine: 3-dose series: • 1st dose: 4-6 months of age • 2nd dose: 4-6 weeks after first dose • 2nd dose dose: 10-12 months of age • Revaccination at 6-month interval

Table 8: Recommended EHV vaccination scheme in Switzerland (Vetsuisse - Konsensus)[241]

Broodmares	**Other adult horses and foals**
Vaccinated (completed BI) • 1st dose: 5. month of gestation • 2nd dose: 7. month of gestation • 3rd dose: 9. Month of gestation	BI: 2-dose series • 1st dose: Generally at the age of 6 months In case of high infection pressure or foals of unvaccinated mares in large farms earliest at the age of 3 months • 2nd dose: 3-6 weeks after the first BI
Not vaccinated before: 3-dose series • Initiation of BI as soon as possible • 2nd dose of BI: 3-6 weeks after the 1st dose • 3rd dose: Booster in the 9. month of gestation if possible, depending on gestation stage	Booster injection: • every 6 months

BI: Basic immunization

Virus shedding after EHV vaccination has not yet been investigated but occurs occasionally following vaccination with a glycoprotein E (gE) deleted bovine herpesvirus 1 (BoHV-1) strain.[242]

1.1.3 Equine Gamma herpesviruses

The subfamily *Gammaherpesvirinae* contains four different generas, named *Percavirus, Macavirus, Rhadinovirus and Lymphocryptovirus*.[8] The genera *Percavirus* include EHV-2, EHV-5, EHV-7 (also called AHV-2) and the closely related asinine herpes viruses AHV-4, AHV-5 and AHV-6.[8,243] Gamma herpesviruses replicate slowly, have a lymphocytic tropism and limited host range.[58] The gamma herpesviruses prefer latency in T and B cells. However, the high seroprevalence to EHV-2 in the equine population relative to the prevalence of the detection of its DNA in peripheral blood lymphocytes (PBL) suggests there are other sites of latency in addition to leucocytes.[244] Cell infection results in cytolytic replication and occurs only in a subset of gamma herpesvirus infected cells.[245] The subfamily of gamma herpesviruses contains more conserved gene regions than the other two subfamilies, but every virus of the subfamily gamma herpesvirus has their own set of unique genes, mostly placed at the terminal region of the genome. These regions are responsible for the individual viral pathogenesis of the virus.[69] Development of viral latency by interference with host immune and inflammatory response is suggested to be associated with distinct genetic sequences, identical to those of eukaryotic cells.[246]

1.1.3.1 Genomic structure

EHV-2 contains a 184 kbp double-stranded DNA genome with a G+C content of 58% and 79 ORF encoding 77 proteins.[247] The EHV-5 genome is shorter, containing 179 kbp but its G+C content is similar.[248] There is a 60% shared identity between the EHV-2 and EHv-5 sequences at DNA and amino acid levels.[12,249] Many genes homologous encoded by both viruses, including DNA polymerase, glycoprotein B (gB) or major capsid protein have been identified.[250] Further similarities between EHV-2 and EHV-5 and γ-EHV contain co-linearity in ORF 74, a marked depletion of CpG dinucleotide and a surplus of TpG + CpA dinucleotide.

The heterogeneity of gamma herpesviruses is in contrast with the homogeneity of alpha herpesviruses. Genomic heterogeneity occurs in both, EHV-2 and EHV-5 and is likely the reason why horses can become infected with different strains over their lifetime.[251,252] In an epidemiologic study of mares and foals held on the same premises, EHV-2 isolates revealed significantly more genomic variations than EHV-5 isolates did.[252] High frequency of recombination among wild strains, even in genomic regions previously characterized stable are detected in various EHV-2 isolated from Australia, Hungary, Germany, England and Sweden.[253] However, by using sequencing of the gB region and restriction fragment length

polymorphism (RFLP), genomic variability was also demonstrated in EHV-5 isolates.[254] Despite these variations in gamma herpesviruses common cytopathic properties are shared.[12]

1.1.3.2 Epidemiology and Transmission

EHV-2 is endemic and the virus has been isolated worldwide.[250,255-258] Reported prevalence in nasal swabs and Peripheral Blood Mononuclear Cells (PBMC) range from 0% - 77%[90,142,257-259] and 30%-99%[90,252,260,261], respectively, depending on geographic location, age and the investigated specimens.

EHV-5 was first identified in 1970 and can be detected in healthy horses as well as horses with respiratory disease.[246] The prevalence of EHV-5 in nasal swabs and PBMC range from 0 - 100%[26,90,142,259,262,263] and 15 – 100%[252,260,261], respectively, depending on geographic location, age and the investigated specimens. To the authors knowledge, the prevalence of EHV-2 and EHV-5 in Switzerland is unknown.

EHV-5 and EHV-2 were both found more often in horses with lower airway inflammation compared to healthy horses.[264] Horses shedding EHV-2 virus were three times more likely to display clinical respiratory disease and is likely to be an important contributor to induce or predispose equids to respiratory disease.[26] Both viruses can simultaneously infect the same animal.[251,255,260]

The most common route of EHV-2 transmission under field conditions is horizontal transfer via nasal and ocular excretion.[265,266] However, the nasal mucosa is suggested as the main reservoir of virus and source of excretion.[256] Investigations on excretion patterns show more common re-infection an re-excretion than constant viral shedding.[265]

Overall, a diagnosis of gamma herpesvirus infection remains difficult to establish, as clinical manifestations and epidemiological distribution patterns are various and unspecific.[246] Horses which test gamma herpesvirus positive during a fever phase with concurrent clinical signs of upper respiratory tract disease should be kept isolated from other horses.[90]

1.1.3.3 Pathogenesis and Clinical signs

The pathogenic potential of EHV-2 in adult horses is suggested to be linked to a modulation of the host immune response.[267] Common virulence mechanisms of gamma herpesviruses include interference with host innate immune response. EHV-2 is able to down-regulate the transcription of equine monocyte chemoattractant protein 1,[267] produce G protein-coupled

receptors[247] and stimulation of T-lymphocytes by macrophages, leading to host hypersensitivity and chronic secondary respiratory disease.[268] The importance of EHV-2 as a co-factor of infection or disease has been reinforced by molecular studies, demonstrating activation of immediate early genes (IEG) of EHV-1 and EHV-4.[116,130] The immediate early proteins encoded by these IEG are central to virus replication and its regulation.

Pathogenicity of EHV-2 and EHV-5 is discussed controversial, as both viruses can be recovered from both clinically affected and healthy animals.[254,269-271] Clinical signs associated with both of these viruses are nasal discharge, enlarged lymph nodes and coughing.[89,261] However, correlation between presence of EHV-2 in tracheal washes from 84 horses in training and respiratory tract disease failed.[272]

EHV-2 has been isolated from foals and adult horses with keratoconjunctivitis or respiratory tract disease. Severe outbreaks of respiratory diseases in foals with 100% morbidity, exhibiting moderate pyrexia, dullness and serous to mucopurulent nasal discharge of several days duration are reported.[273,274] Other diseases associated with natural EHV-2 infection include granulomatous dermatitis[275] and abortion.[276,277] However, experimental inoculation of equine fetuses failed to infect fetus or induce abortion.[278] Investigations on duration of EHV-2 detection following experimental intranasal EHV-2 inoculation in a murine infection model showed virus recover up to 30 days from lung, trachea, nasal turbinates and trigeminal ganglia. However, long-term latent infection of B lymphocytes was not achieved in this model.[279,280]

EHV-5 is associated with Equine pulmonary fibrosis (EMPF), a rare interstitial lung disease affecting horses at all ages.[19,20,281-294] Pathogenesis is not completely elucidated, herpesviruses are suspected to be involved in pulmonary fibrosis in humans, rodents, and domestic animals (dog, cat, horse).[295,296] There are similarities between fibrotic disease in these species and EMPF in horses regarding the age at clinical onset and progression of the disease as well as poor response to therapy.[297] Why some horses develop signs of EMPF while others remain healthy is unclear. Clinical signs of EMPF depend on the stage of disease and include tachypnoea, increased respiratory effort, tachycardia, weight loss/poor overall condition, intermittent fever and cough.[20,298] Long term prognosis for this horses remains fair to poor.[282]

AHV-4, AHV-5 and AHV-6 have been detected in donkeys with interstitial pneumonia, with lesions similar to EMPF.[243,299] Detection of AHV-5 in horses is rarely described. AHV-5 was detected in horses with respiratory disorders or “poor performance syndrome”.[246] Concurrent detection of EHV-5 and AHV-5 in an EMPF case has been described in a case report.[283] AHV-5 has also been detected in lung tissue of a horse with pyogranulomatous pneumonia, a pathology differing from described EMPF cases in horses and interstitial pneumonia in donkeys with AHV-5 infection.[300]

1.1.3.4 Diagnosis of infection

EHV-2 and EHV-5 can be diagnosed by indirect methods, such as complement fixing test (CFT), neutralizing tests and ELISA as well as cell culture and DNA amplification by PCR technique. An ELISA that can discriminate between EHV-2 and EHV-5 has been developed[256] and a blocking ELISA validated for EHV-2 serodetection is available.[301] Cell culture are performed by isolation from the PBL of infected horses using co-culture techniques[252] with added IL-2 to increase sensitivity of detection.[261] CPE of EHV-2 are usually detected within 12 – 21 days,[274] compared to 4 – 12 days with applied co-cultivation technique.[252] Slow development of CPE with evidence of ballooning over 3 – 4 passages to increase amount of available virus is reported in EHV-5 culture.[20] Multiplex-PCR has been used in epidemiological studies for EHV-2 and EHV-5.[302] Most techniques target the gB gene region but the the gH region is also commonly used.[260]

1.1.4 Aims of the Study

The objectives of this study were:

- To follow the antibody response following EHV-1 and -4 vaccination using different vaccination schemes
- To evaluate post-vaccination virus shedding
- To investigate prevalence of gamma herpes virus shedding in different regions of Switzerland

2 Material and Methods

2.1 PART A - EHV-1 and EHV-4 shedding and antibody response after vaccination

The study was performed under the regulations of the Swiss federal authorities for animal experimentation (Animal use license no. ZH006/18).

2.1.1 Farms and animals

24 horses were included in the study, including horses that were vaccinated (n = 18, Group A-C) and horses that were left unvaccinated (controls, n = 6, Group D). Geographical origin, number of horses and vaccination or control status as part of the study as well as vaccination history are displayed in Table 9.

Table 9: Summary of included farms and animals

Farm	Canton	Group	n	Vaccination Protocol (A-D)	Vaccination history
1	AG	Vaccination	3	A	BI > 4 years ago Booster q 6months
2	AG	Vaccination	11	B (n = 5) and C (n = 6)	None in the last 2 years
3	SO	Vaccination	3	A	BI > 4 years ago Booster q 6months
4	Germany	Vaccination	1	C	None in the last 2 years
5	ZH	Control	6	D	Never vaccinated before

BI: Basic immunization (two vaccines administered four weeks apart)
Group A: Booster was continued q 6 months throughout the study
Group B: Vaccinated two times, six months apart without prior BI
Group C: BI followed by a booster after six months
Group D: No vaccination as part of the study

Horse owners were contacted through personal contacts of the researchers, recent patients and their stable mates at the University of Zurich Teaching Hospital and referring veterinarians. Horses were included in the study if owners provided written consent. Inclusion criteria for control horses were no EHV-1/4 vaccination history in the last two years and an informed decision of the owner to continue management of the horses without EHV vaccination for the next 12 months. Inclusion criteria for vaccinated horses were willingness of the owner to

continue the current vaccination protocol for at least 12 months (Group A) or willingness to agree to a new vaccination protocol in unvaccinated horses (Group B and C). Not all horses present on the farms were included in the study due to missing owner consent. Samples were collected on farm and the routine management of the horses was not changed.

There was movement of horses off and on all farms during the whole sampling period. The herds were therefore not considered closed herds. Reason for animal movement included start at competition (Farm 2, 5), additions to the herd (Farm 2, 3, 4, 5), removal of animals from the herd (Farm 1, 2, 4, 5) and hospitalization at an equine clinic (Farm 4). On Farm 2 and 5, a pregnant mare vaccinated against EHV-1,4 was in contact with the sampled horses. The mare of Farm 2 foaled in the last third of the second sampling period whereas in Farm 5 EHV-1 induced abortion in the last third of the first sampling period occurred. During the first sampling period, an outbreak of strangles was diagnosed in Farm 3, including two horses of the study population.

An EHM outbreak had occurred on the farm of Group B and C in 2016, two years before sampling of the study was started. Horses that were present at the time of the outbreak (n = 4) received EHV-1/4 basic immunization (BI, two vaccines administered four weeks apart) followed by a booster after six months. After that, vaccination was discontinued.

2.1.2 Vaccination

All vaccinations were performed with Duvaxyn®EHV-1,4 ad us.vet. (Zoetis Schweiz GmbH, Delémont), containing one vaccination dose of 1.5ml solution. The groups were vaccinated as shown in Table 10.

Table 10: Vaccination scheme of each group

Group	Median age (range)	Breed (n)	Vaccination History	BI as part of the study	Summer vaccination (July-Sep)	Winter vaccination (Dec-March)
A n = 6	14.5 (4 - 21)	WB (3) AA (2) FR (1)	BI > 4 years ago Booster q 6m	No	Yes	Yes
B n = 5	5 (2 - 13)	WB (5)	No vaccination within last 24m	No	Yes	Yes
C n = 7	11 (7 - 23)	WB (5) T (2)	No vaccination within last 24m	Yes	Yes	Yes
D n = 6	12.5 (7 - 17)	WB (6)	Never vaccinated	No	No	No

BI: basic immunization, consisted of 2 vaccines given 4 weeks apart
WB: Warmblood, AA: Anglo-Arabian, FR: Friesian, T: Tinker
m: months

2.1.3 Sampling

An overview of the sampling scheme for blood and nasal swabs are shown in Figure 3 and 4, respectively. All horses in Group A and B were studied for a total of 11 months, starting immediately before the first vaccination in summer, to five months after the 2nd vaccination in winter. Horses in Group C received an additional vaccine due to basic immunization (BI) with two doses 4 weeks apart followed by the booster and were therefore followed for a total of 12 months.

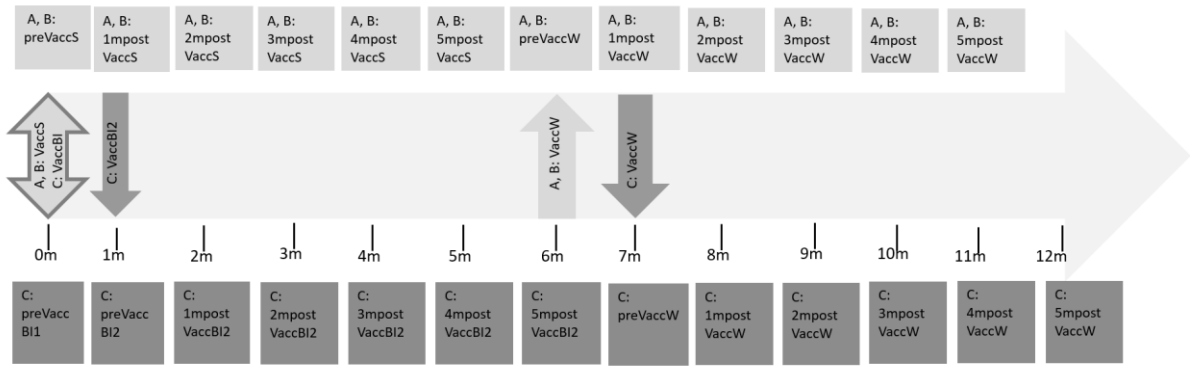
Samples for serologic analysis were obtained at 12 time points from horses in Group A and B. The time points were as follows: before the summer vaccination and 1, 2, 3, 4, 5 months after the summer vaccination, immediately before the winter vaccination and again 1, 2, 3, 4, 5 months after the winter vaccination. Horses of Group C were sampled before part one and two of the BI and 1, 2, 3, 4, 5 months after the second part of the BI as well as before winter vaccination and 1, 2, 3, 4, 5 months after winter vaccination. Therefore, an additional sample 1 month after the second part of the BI was obtained for this group. Sampling of horses from Group D started at a later starting point, followed by the same protocol as Group A and B, but no vaccination was performed. The first taken blood sample of every horse in this study is defined as the pre-study status. At each time point, 5ml of blood was collected by venipuncture of the jugular vein with a Vacutainer and a Vacuette® tube, containing Serum Sep Clot Activator (Greiner Bio-One International). Samples were processed within 12 hours. Serum was allowed to stand for at least one hour and afterwards centrifugated with a Hermle LAB-Z200 A centrifuge for 10 minutes at 3000 rpm. Serum was then poured off in a sample tube and stored in a -80°C freezer.

To detect EHV-1 and EHV-4 shedding, a nasal swab (eNAT™ ® Copan Diagnostics Inc.) was collected from every horse before summer and winter vaccination and daily for 5 days after summer and winter vaccination. Horses in Group C were sampled before part 1 and 2 of the BI and daily for 5 days after BI as well as before winter vaccination and daily for 5 days after winter vaccination. Sampling of horses from Group D started at a later starting point, followed by the same protocol as Group A and B, but no vaccination was performed. The first taken nasal swab of every horse in this study is defined as the pre-study status. Nasal swabs were immediately inserted into the virologic medium (Copan eNAT flocked swab 606CS01R). Samples were stored in a freezer at -80°C.

All serum samples and the nasal swabs prior to vaccination of Group A, B and C were obtained by the first author of this study except for one horse on Farm 4 (Group C) from which all samples were taken by the owner (veterinarian), and all horses on Farm 2 (Group A) from which samples during the second sampling period were taken by the owner (veterinarian technician). The nasal swabs after vaccination were obtained by the owners, nasal swabs were provided to the owners for collection.

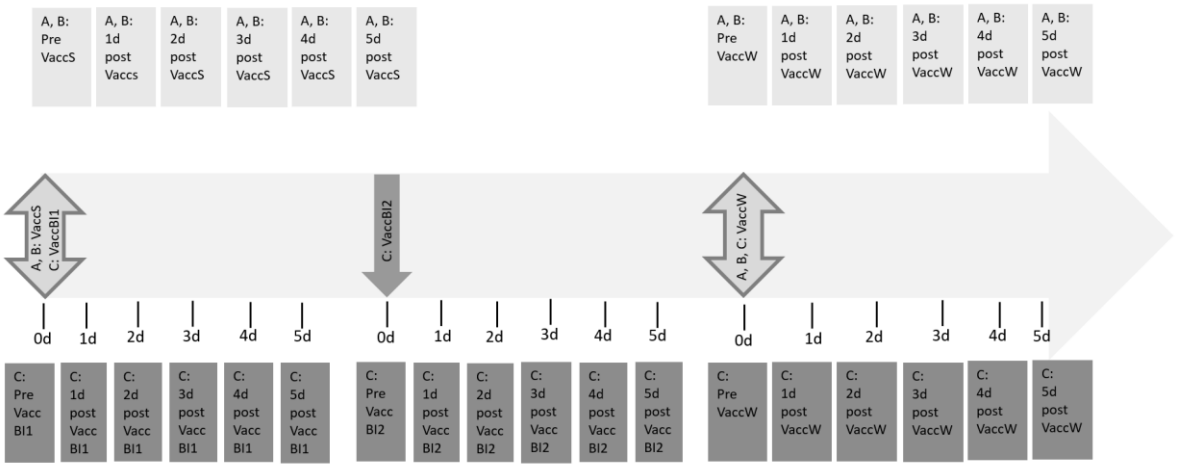
All serum and nasal swabs samples of Group D were taken by the barn veterinarian as per wishes of the owners.

Figure 3: Schematic representation of sampling timepoint for serological analysis in 18 horses vaccinated against EHV-1 and EHV-4



m: months; Vacc: Vaccination; preVacc: pre vaccination; mpostVacc: months post vaccination
Group A and B received 2 vaccines; a summer and a winter vaccine six months apart (VaccS and VaccW), Group C was administered 3 vaccines, including a two-part BI 4 weeks apart (VaccBI1, VaccBI2) and a winter vaccine (VaccW). In Group C an additional blood sample (12m) were collected due to the additional vaccine. The booster immunization of Group C lead to a shift of the sampling timepoints compared to the scheme of Group A and B. Group D was sampled according to the same protocol as Group A and B, but no vaccination was performed.

Figure 4: Schematic representation of sampling timepoints for evaluation of nasal shedding of EHV-1 and EHV-4 in 18 vaccinated horses



d: days; Vacc: Vaccination; preVacc: pre vaccination; dpostVacc: days post vaccination
Group A and B received 2 vaccines; a summer and a winter vaccine six months apart (VaccS and VaccW), Group C was administered 3 vaccines, including a two-part BI 4 weeks apart (VaccBI1, VaccBI2) and a winter vaccine (VaccW). In Group C six extra nasal swabs (preVaccBI2, 1-5dpostVaccBI2) were collected due to the additional vaccine. Group D was sampled according to the same protocol as Group A and B, but no vaccination was performed.

2.1.3.1 Sampling procedure

Samples were taken without wearing gloves and always in the same order, starting with the nasal swab, followed by obtaining the blood samples. At the time points when the vaccine was administered the chronology of sampling was as follows:

1. Completion of the passport of every horse including transfer of the sticker from the vaccination bottle into the passport.
2. Preparation of the vaccine by drawing the liquid into a syringe
3. Sample collection (nasal swab then blood sample)
4. Administration of the vaccine.

After results became available an additional experiment was performed to evaluate potential contamination of the nasal swab with EHV-1 and EHV-4 DNA during the sampling procedure. An EHV-1,4 vaccine vial (Duvaxyn®EHV-1,4, Zoetis) and a tetanus vaccine vial (Equilis®Te, MSD) were investigated for presence of EHV-1/4 DNA on the surface of the vaccine bottle. Before starting the trial, gloves of the researcher were sampled to rule out previous contamination. The outside of the Duvaxyn vaccine vial and Tetanus vaccine vial as well as the gloves of the researcher after handling the bottles to remove the sticker and draw the liquid into the syringe were swabbed with eNAT swabs. The content of both vaccine bottles was tested with two different swabs, eNAT (eNAT$^{TM\,®}$ Copan Diagnostics Inc) and UTM (UTM® Copan Diagnostics Inc), these samples served as positive and negative controls. The eNAT swabs contain a medium to preserve nucleic acid, therefore suitable for

genome detection. The UTM swab conserves and maintain virus particles infectious, therefore appropriate for virus isolation on cell culture but also useable for genome detection. Both swabs were used to exclude contamination of the swab or medium leading to false positive results as well as to assess, if a similar PCR reaction result is achieved with both swabs contaminated by the vaccine virus. The gloves were changed after each step to control every individual operation. The samples were analyzed immediately following the same procedure as the nasal swabs.

2.1.4 DNA detection of EHV-1 and EHV-4 (real-time PCR)

DNA was extracted with a commercial Qiamp DNA mini kit (Qiagen, city), according to the manufacturer's instructions. Real-time PCR was performed to test for presence and quantity of EHV-1 and/or EHV-4 and a housekeeping gene in the sample. Two PCR reaction mixes, one multiplex PCR for EHV (Tab. 11) and one for equine-12S (eq-12S; Tab. 12) as the housekeeping gene were prepared. The master mixes consisted of the TaqMan® Universal PCR Master Mix (Applied Biosystems; Tab. 11 and 12). The EHV-1 and EHV-4 specific primers and probes targeting glycpoprotein B used in this study have been previously described.[303] The eq-12S primers and probes were designed in-house for a prior study using primerExpress software (ThermoFisher) according to Table 13. Extracted DNA of an EHV-1 and an EHV-4 infected horse in 1:10 dilution were added as positive controls. The Eq-12S positive control was used in a dilution of 1:100 and Diethylpyrocarbonate (DEPC) treated water was used as a negative control.

PCR was performed in 45 cyles with the QuantStudio 7 real-time PCR machine. Cycling conditions are shown in Table 14. The results of the real-time-PCR were reported as CT-values.

Table 11: PCR Master Mix EHV-1/4

EHV-Mix (number of samples + 5 controls + 1)	**1x sample (µl)**
2x Universal Mix	10
EHV-1 F primer (20uM)	0.4
EHV-1 R primer (20uM)	0.4
EHV-1 probe-FAM (10uM)	0.2
EHV-4 F primer (20uM)	0,8
EHV-4 R primer (20uM)	0,8
EHV-4 probe-VIC (10uM)	0.4
Nuclease-free water	2
Final volume mix	15
DNA	5

F: Forward, R: Reverse

Table 12: PCR Master Mix eq12S

eq12S-Mix (number of samples+3controls+1)	**1x sample (µl)**
2x Universal Mix	10
e12S F primer (10um)	1
e12S R primer (10um)	1
e12S probe (5um)	1
Nuclease-free water	5
Final volume mix	18
DNA	2

F: Forward, R: Reverse

Table 13: Primer sequences for EHV-1/4 and eq12S PCR

Name	Sequence 5'->3'	Start	End	Reference
EHV-1 F primer	CATGTCAACGCACTCCCA	1247	1264	D00401.1
EHV-1 R primer	GGGTCGGGCGTTTCTGT	1293	1309	D00401.1
EHV-1 probe	FAM-CCCTACGCTGCTCC-MGB	1277	1290	D00401.1
EHV-4 F primer	GGGCTATTGGATTACAGCGAGAT	2571	2593	M26171.1
EHV-4 R primer	TAGAATCGGAGGGCGTGAAG	2610	2629	M26171.1
EHV-4 probe	VIC-CAGCGCCGTAACCAG-MGB	2595	2609	M26171.1
e 12S F primer	GGAGCCTGTTCCATAATCGATAA	555	577	X79547.1
e12S R primer	GTTTGCTGAAGATGGCGGTATAT	638	616	X79547.1
e12S probe	FAM-TAAACCCCACCATCCCTTGCTAATTC AGC-TAMRA	585	613	X79547.1

Primer sequences for EHV-1/4 and eq12S PCR used to detect viral shedding before and after vaccination of 18 vaccinated horses and 6 unvaccinated controls.
F: Forward, R: Reverse

Table 14: Cycles of PCR.

	Temp. °C	Duration	Unit	Cycles
Hold Stage	50	2	min	
	95	10	min	
PCR Stage	95	15	sec	45x
	60	60	sec	

2.1.5 EHV-1 and EHV-4 antibody detection (ELISA)

The sera were tested using a commercially available ELISA Kit (Svanovir EHV1/EHV4-Ab ELISA kit, company, city) for presence and concentration of antibodies against EHV-4 and/or EHV-1 according to the manufacturer's instructions. The optical density (OD)-values were measured in a photometer at 450 nm. OD-values of the EHV-1 and EHV-4 coated wells from each sample were corrected with the value of the respective negatively coated well according to the manufacturer's instructions, resulting in "corrected OD-values":

'$OD_{EHV1} - OD_{Control\ Antigen} = OD_{corr\ EHV1}$; $OD_{EHV4} - OD_{control\ Antigen} = OD_{Corr\ EHV4}$'

For validity of the test, the controls were assumed as: "OD_{corr} Positve Control > 0,6 and OD_{corr} Negative Control < 0,1". A sample was considered positive if OD_{corr} is > 0,2 and negative if OD_{corr} is < 0,1. An OD_{corr} between 0,1 - 0,2 was considered doubtful;[92,304] the manufacturer recommends retesting of the animal at a later time point in these cases. As retesting was not possible in this study, we designated these questionable results as non-interpretable (nip) and elected to present them separately. A peak in serological response was defined by the author of the study as a raise in titers of ≥ 0.5 OD-Values.

2.1.6 Statistical analysis

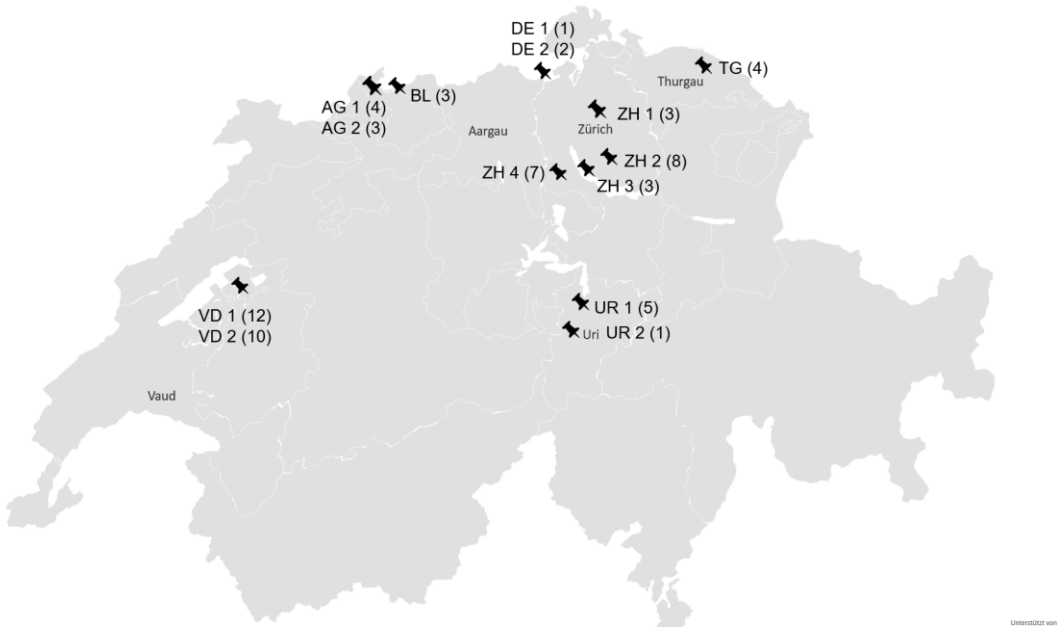
Descriptive statistics were used to report antibody prevalence and virus shedding in relation to vaccination.

2.2 Part B – Screening for equine gamma herpesviruses in healthy horses

2.2.1 Farms and animals

Previously extracted DNA from 68 nasal swabs collected as part of a different project in 2017 were used. The nasal swabs originated from healthy horses from six Swiss cantons (Baselland, Thurgau, Uri, Zurich, Aargau, Waadt) from twelve farms (BL, TG, UR 1, UR 2, ZH 1, ZH 2, ZH3, ZH4, AG1, AG2, VD1, VD2) and from horses from two farms (DE1, DE2) with an EHV-1 outbreak in Germany (Fig. 5).

Figure 5: Geographical distribution of the stables of horses screened for shedding of equine gamma herpesviruses



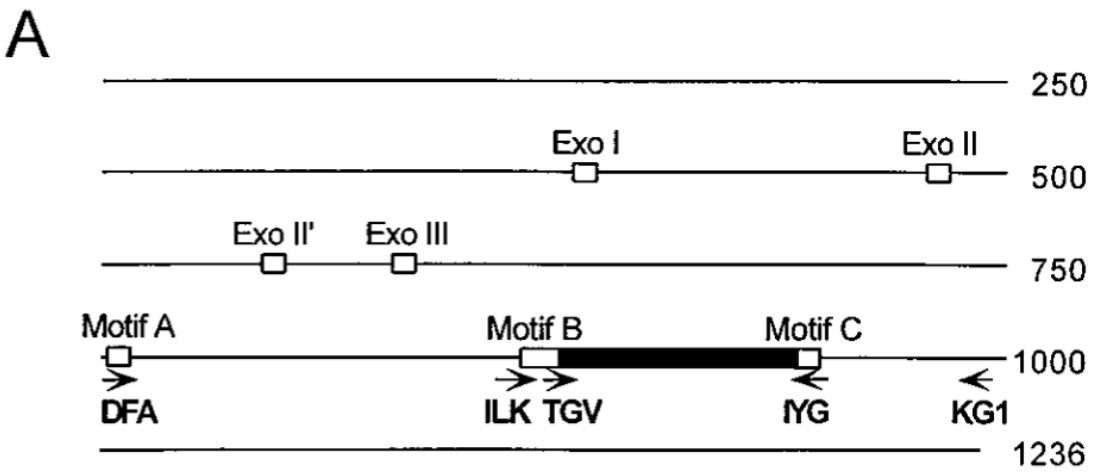
AG: Aargau; BL: Baselland; DE: Germany; TG: Thurgau; UR: Uri; VD: Waadt; ZH: Zurich
ZH 1 (3): Zurich, barn number, number of sampled horses per barn

DNA extraction from the nasal swabs was performed as part of a different project using a commercial DNA extraction kit (Qiamp DNA mini kit, Qiagen, city) according to the manufacturer's instructions. DNA was stored at –80°C and had only undergone one thawing cycle prior to use in this project.

2.2.2 Panherpes nested PCR

A Panherpes nested PCR was performed as previously described.[305,306] This PCR targets a highly conserved region of the herpesviral DNA polymerase gene. As degenerate consensus primers are used, the PCR is capable of detecting known and novel herpesviruses without prior information on DNA sequence.[307] This nested format with two forward (DFA and ILK) and one reverse (KG1) degenerate primer in the first PCR and one forward (TGV) and one reverse degenerated primer (IYG) in the second PCR is shown in Figure 6.[305] Primer sequences are shown in Table 11.[305] The expected size of the product after Panherpes nested PCR is 215-235 bp.[306]

Figure 6: Relative position of the PCR priming sites in the nested format (VanDevanter, 1996)[305]



Relative positions of PCR priming sites for DFA, ILK, KG, TGV, and IYG primers (arrows) within an amino acid-coding map of the human herpesvirus 1 DNA polymerase gene [305]. Previously described [308] coding motifs conserved across broad classes of DNA-dependent DNA polymerases (Exo I, II, II9, and III and motifs A, B, and C) are noted with open boxes. The region obtained by nested consensus primer PCR with the indicated primers is highlighted with a black box. The numbers identify amino acid positions [305].

Table 15: Primer sequences for Panherpes nested PCR (VanDevanter, 1996)[305]

PCR	Primer	Primer Name	Sequence
1.	Forward	DFA	5'- GAY TTY GCN AGY YTN TAY CC-3'
	Forward	ILK	5'- TCC TGG ACA AGC ARN YSG CNM TNA A-3'
	Reverse	KG1	5'- GTC TTG CTC ACC AGN TCN ACN CCY TT-3'
2.	Forward	TGV	5'- TGT AAC TCG GTG TAY GGN TTY CAN GGN GT-3'
	Reverse	IYG	5'- CAC AGA GTC CGT RTC NCC RTA DAT-3'

A minor modification to the reaction mixes described by Ehlers et al. (1999)[306] was made. In the first PCR round, 5 µl of extracted sample DNA were used and for the second PCR round 1µl of the product from the first round was applied. The final volume of both mixtures added up to 25µl and contained 2.5µl PCR Buffer (10x, Qiagen, Hombrechtikon, Switzerland), 200µM of each deoxynucleotide triphosphate, 1µM of each primer and 2 units HotStarTaq DNA Polymerase (5U/µl, Qiagen, Hombrechtikon, Switzerland). DEPC treated water was added as needed to reach the final reaction volume of 25 µl. DEPC treated water was used as negative control and DNA extracted from a cow with confirmed bovine malignant catarrhal fever (BMCF) was used as a positive control.

The Peltier Thermal Cycler-200 (MJ Research) was used for thermal cycling according the protocol of Ehlers et al. (1999)[306] with a minor modification. The initial denaturation time was 12 minutes at 95°C instead of 3 minutes at 95°C. Before loading the products from the second PCR on a 2% agarose-gel, containing Gel Red (1000x, Biotium, Hayward CA, USA), 5µl of Orange Loading Dye (6x, Thermo Fisher Scientific, Waltham MA, USA) was added. A 50bp DNA Ladder (New England Biolabs) was used. Electrophoresis took approximately two hours at 100V. Visualization of bands with the expected size (215 - 235bp) was performed under UV light.

Positive samples, except the positive control, were cut from the gel with a sterile scalpel blade. DNA was extracted from the Gel with a QIAquick® Gel Extraction Kit according to the manufacturer's instructions (Qiagen, Hombrechtikon, Switzerland). Elution of the DNA was performed with 30µl of elution buffer, followed by the third PCR applying non-degenerated primers (Tab. 12), to determine which herpesvirus was present.

Table 16: Primer sequences for the third (sequencing) PCR (VanDevanter, 1996)[305]

Primer	Primer Name	Sequence
Forward	TGVseq	5'- CAT CTG ATG TAA CTC GGT GTA-3'
Reverse	IYGseq	5'- GAC AAA CAC AGA CTC CGT-3'

The reaction mixture for the third PCR was similar to the one delineated for the second PCR described above. The only exception was the use of 200 µM of each sequencing primer and 1 unit of HotStarTaq DNA Polymerase (5 U/µl). A commercial QIAquick® PCR Purification Kit (Qiagen, Hombrechtikon, Switzerland) was used to purify DNA and subsequent eluting with 30 µl of elution buffer was performed. The total amount of amplified DNA was measured in Nanodrop (ND-1000 Spectrophotometer, Scientific AG, Kloten, Switzerland). Of each positive sample 45 ng of DNA was mixed with 3 µM of sequencing primer forward (10 µM, 5´CAT CTG ATG TAA CTC GGT GTA 3´) and filled up with DEPC to a final volume of 15 µl. Sequencing was performed by Microsynth (Balgach, Switzerland) and analysis was performed with NCBI BLAST® (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Samples were determined to be positive if a band was visible in the expected size in the agarose-gel with or without a high identity to herpesviral sequences deposited in GenBank.

2.2.3 Statistical analysis

Descriptive statistics were used.

3 Results

3.1 PART A – EHV-1 and EHV-4 shedding and antibody response after vaccination

3.1.1 Animals

Of the initially included 24 horses (18 vaccinated and 6 controls), 20 completed the study (15 vaccinated and 5 controls). Two horses were sold (Group A and B), one horse moved abroad (Group D), and one horse was euthanized due to chronic lameness during the study period (Group C). In one horse of Group C (H21), the period between the basic immunization was 2 months instead of the planned one month, as the horse was hospitalized when the second BI was planned. This horse remained in the study.

Four horses (2 of Group A, 1 of Group B, 1 of Group C) had all serum and nasal swabs available. Six serum samples from six horses (Group B, and D) were not collected due to one horse being absent at a competition at the sampling time (n = 1/6), and human error (n = 5/6). The latter contained all samples of the control Group at beginning of the winter sampling period. Forty-eight nasal swabs of 16 horses (Group A, B, C and D) could not be analysed due to missing samples from horses who were absent from the barn at the sampling time point (n = 9/48), human error resulting in an incorrect sampling timepoint (n = 30/48) or sampling material (n=4/48) and lack of cooperation (n = 5/48). Incorrect sampling material was obtained when nasal swabs were inserted into the wrong transport medium due to human error. Sampling at an incorrect timepoint occurred due to human error resulting in monthly sampling instead of daily sampling after vaccination of the first three horses. A more detailed presentation of all missing serum samples and nasal swabs that could not be analysed is presented in Table 17. An overview of all available samples from every horse is presented in table 18 a-c (see Appendix).

Table 17: Overview of missing samples from horses (n=20) included in a study on nasal shedding and antibody kinetics after vaccination against EHV-1 and EHV-4

Horse (n = 20)	Group	Missing nasal swabs (n = 48)	Missing serum samples (n = 6)	reason
H1	D	1	1	competition (NS) incorrect sampling timepoint (S)
H2	D	1	1	competition (NS) human error (S)
H3	D	1	1	competition (NS) human error (S)
H4	D	1	1	competition (NS) human error (S)
H6	D	1	1	competition (NS) incorrect sampling timepoint (S)
H7	C	1	0	incorrect sample material
H8	C	5	0	lack of cooperation
H9	C	1	0	incorrect sample material
H10	C	0	0	all samples available
H11	C	1	0	incorrect sample material
H12	B	6	0	incorrect sampling timepoint
H13	B	6	0	incorrect sampling timepoint
H14	B	6	0	incorrect sampling timepoint
H16	B	0	0	all samples available
H17	B	1	0	competition
H18	B	3	1	competition (NS and S)
H19	B	1	0	incorrect sample material
H21	C	12	0	incorrect sampling timepoint
H22	A	0	0	all samples available
H23	A	0	0	all samples available

NS: nasal swabs; S: Serum samples; H1-21: Horse designation
Group A: BI >4 years ago, q6months booster which continued throughout the study
Group B: No vaccination history, vaccinated two times, six months apart without prior BI
Group C: No vaccination history, BI in summer followed by a booster after six months
Group D: No vaccination history and no vaccination as part of the study, natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS

3.1.2 Adverse reaction to the vaccine

One horse in Group C (H7) showed an adverse reaction after administration of the first vaccine. Swelling at the injection side with a diameter up to 10cm, elevation of the rectal temperature up to 40°C for four days, reduced general condition, inappetence and stiff gait were recorded. This horse was present but not clinically affected during the EHM outbreak in 2016 and received basic immunization following the outbreak without recorded adverse reaction at the time.

3.1.3 EHV outbreak in control Group D

Abortion due to EHV-1 infection occurred in the population of the control group (Group D) 14 weeks after starting the study. The affected mare was not part of the study but in close contact to the sampled horses due to group housing of the animals. After confirmation of EHV-1 abortion, the mare was separated from the rest of the group. There were no other pregnant mares on the farm at the time and none of the other horses developed clinical signs. Sampling was continued following the reported outbreak according to initial study design. Instead of considering this group an unvaccinated control Group, the Group was now considered as naturally exposed.

3.1.4 Serological EHV-1 response after vaccination

Of a total of 240 samples (Group A-D), 80 Samples were positive, and 135 samples were negative and 25 were considered non interpretable (nip). Thirteen nip results were collected from vaccinated horses of Group B and C, another 12 from control Group D.

3.1.4.1 Pre-study antibody status

All serological results prior to the first vaccination of the study protocol (Group A – C) and start of sampling in the control group are defined as pre-study status. Overall, 5/18 (28%) of horses had a positive pre-study status, 2/5 were in Group A with a regular vaccination history, and one each in Group B - D without prior vaccination in the last 2 years. A positive pre-study status was more common in horses from in Group A which received the BI more than 4 years ago and booster vaccinations every six months since (including during the study period) compared to horses that were not vaccinated (Group B - D). Pre-study status of control Group was comparable to Group B and C.

3.1.4.2 Serological results of sample groups

After vaccination 8/15 (53%) of horses were seropositive on at least one timepoint, and 3/15 (20%) were positive at all time points. In Group D which was naturally exposed during the outbreak 3/5 (60%) of horses were positive on at least one time point after the exposure and 1/5 (20%) were positive at all timepoints after the exposure. EHV-1 seroprevalence based on vaccination group and timepoint is shown in Table 19.

After both vaccinations Group A and B had a higher seroprevalence than Group C, despite the fact that horses in group C were vaccinated with a BI followed by boosters. However, 10/25 nip results were collected from horses of Group C., including one horse of this Group showing 6 nip results. In horses of Group A, no nip results were measured.

A higher seroprevalence was seen in Group D 8 weeks after the outbreak occurred (sampling time point 5mpostVaccS/5mpostVaccBI2) compared to Group A-C. Seroprevalence however dropped 8 weeks later (sampling time point 1mpostVaccW) and was lower to the vaccinated Groups A and B, but higher than Group C.

Table 19: EHV-1 seroprevalence in 20 horses vaccinated against EHV-1/4 with three different protocols or exposed to EHV-1 at various timepoints

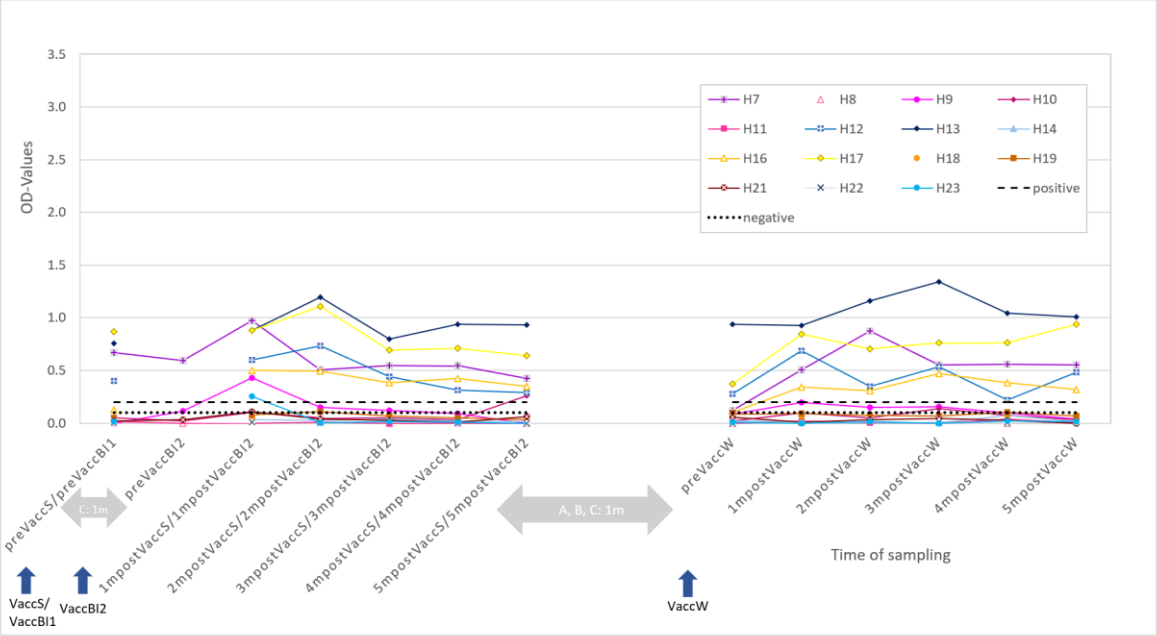
EHV-1 **Timepoint of sampling**	**A (n = 5)**	**B (n = 4)**	**C (n = 6)**	**D (n = 5)**	**Total A-C (n = 15)**
preVaccS/preVaccBI1	40% (2/5)	33% (1/3)	17% (1/6)	25% (1/4)	29% (4/14)
preVaccBI2			25% (1/4)		
1mpostVaccS/1mpostVaccBI2	60% (3/5)	50% (2/4)	50% (2/4)	50% (2/4)	54% (7/13)
2mpostVaccS/2mpostVaccBI2	40% (2/5)	67% (2/3)	20% (1/5)	0% (0/4)	38% (5/13)
3mpostVaccS/3mpostVaccBI2	40% (2/5)	67% (2/3)*	20% (1/5)	50% (2/4)	38% (5/13)
4mpostVaccS/4mpostVaccBI2	40% (2/5)	67% (2/3)	17% (1/6)	67% (2/3)**	36% (5/14)
5mpostVaccS/5mpostVaccBI2	40% (2/5)	50% (2/4)	33% (2/6)	75% (3/4)	40% (6/15)
preVaccW	40% (2/5)	25% (1/4)	0% (0/5)	missing samples	21% (3/14)
1mpostVaccW	40% (2/5)	50% (2/4)	20% (1/5)	25% (1/4)	36% (5/14)
2mpostVaccW	40% (2/5)	50% (2/4)	20% (1/5)	50% (2/4)	36% (5/14)
3mpostVaccW	40% (2/5)	50% (2/4)	25% (1/4)	40% (2/5)	38% (5/13)
4mpostVaccW	40% (2/5)	50% (2/4)	17% (1/6)	50% (2/4)	33% (5/15)
5mpostVaccW	40% (2/5)	50% (2/4)	17% (1/6)	50% (2/4)	33% (5/15)

preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination;
preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
mpostVaccBI2: months after the second dose of the basic immunization (Group C only);
BI: Basic immunization consisting of two doses given 4 weeks apart
Group A: BI >4 years ago, q6months booster which continued throughout the study
Group B: No vaccination history, vaccinated two times, six months apart without prior BI
Group C: No vaccination history, BI in summer followed by a booster after six months
Group D: No vaccination history and no vaccination as part of the study, natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS
*The total number of animals varies due to one missing serum sample
** First sample 2 weeks after an outbreak of EHV-1 occurred in the barn
The total number of animals per group varies for each timepoint as non-interpretable results were removed

3.1.4.3 Serological results of single animals

In the vaccinated Groups A-C 8/15 (53%) horses showed a serological response to vaccination in summer. A sustained rise after vaccination over the whole sampling period was seen in 5/8 (63%) of the seropositive horses, the other three animals (38%) were positive only at one single sample timepoint (Fig. 7). After winter vaccination 5/15 (33%) showed a response, and all of them showed a sustained response over the whole period.

Figure 7: Longitudinal EHV-1 antibody concentrations in horses vaccinated against EHV-1/4 with different protocols (n = 15)



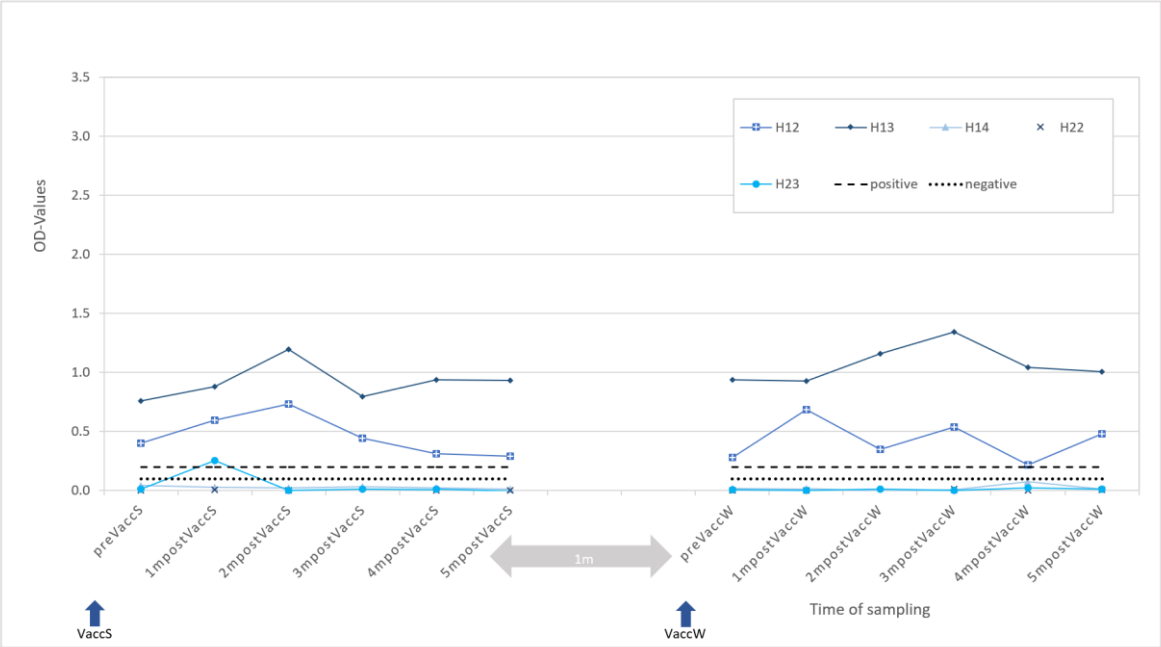
H: Horse number; BI: Basic immunization consisting of two vaccine doses administered 4 weeks apart (VaccBI1, VaccBI2); VaccS: summer vaccination; VaccW: winter vaccination, preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination, preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only); preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only), mpostVaccBI2: months after the second dose of the basic immunization (Group C only); OD: Optical density
Shades of blue: Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study; Shades of yellow: Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI; Shades of purple: Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

In the following figures (Fig. 8 - 11) individual serological response of every horse is shown based on vaccination group.

3.1.4.4 Group A – Basic immunization > 4 years ago with boosters every 6 months

In group A 2/5 (40%) of horses showed a sustained high EHV-1 antibody concentration (OD-Value > 0.2) over the whole sampling period. One horse was seropositive at one timepoint (one month after summer vaccination). The remaining two horses were negative at every timepoint after winter and summer vaccination (Fig. 8).

Figure 8: Longitudinal EHV-1 antibody concentrations of horses in Group A (regular pre-study vaccinations) which received a summer and winter vaccination during the study (n = 5)

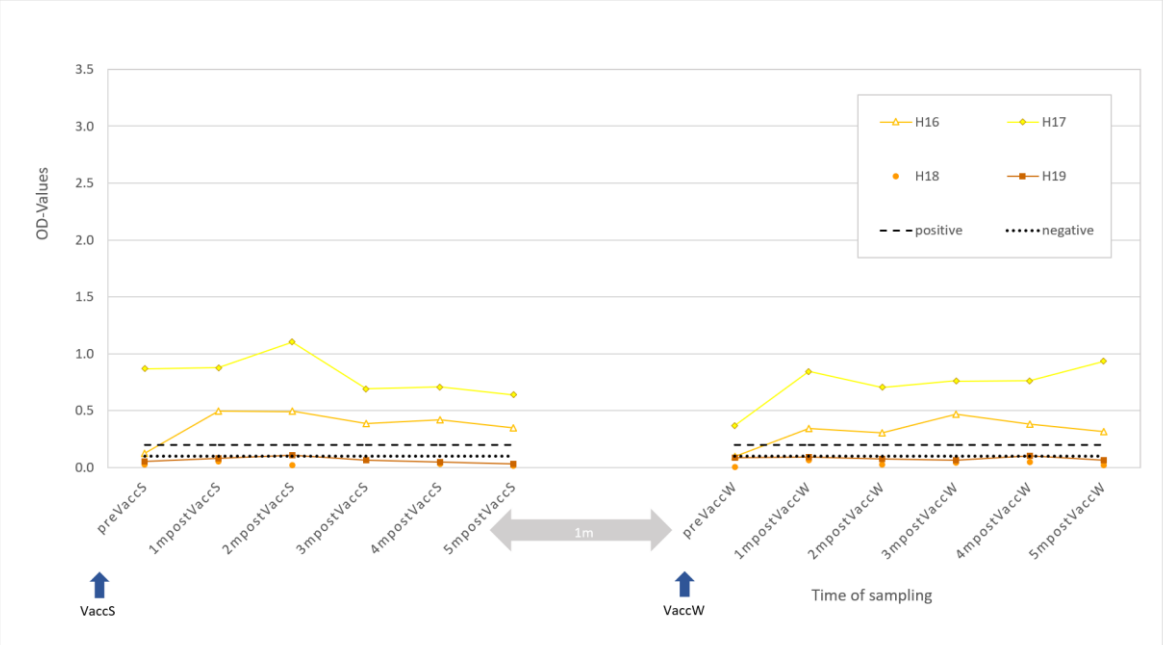


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.4.5 Group B – no vaccination history, vaccinated twice 6 months apart

In Group B 2/4 (50%) horses showed a serological response to both vaccinations and remained positive over the whole sampling period. One of them had a positive EHV-1 antibodies pre-study status, the other did not. The remaining two horses had a negative pre-study status and did not show a serological response to either vaccination (Fig. 9).

Figure 9: Longitudinal EHV-1 antibody concentrations of horses in Group B (no pre-study vaccinations) which received a summer and winter vaccination during the study (n = 4)

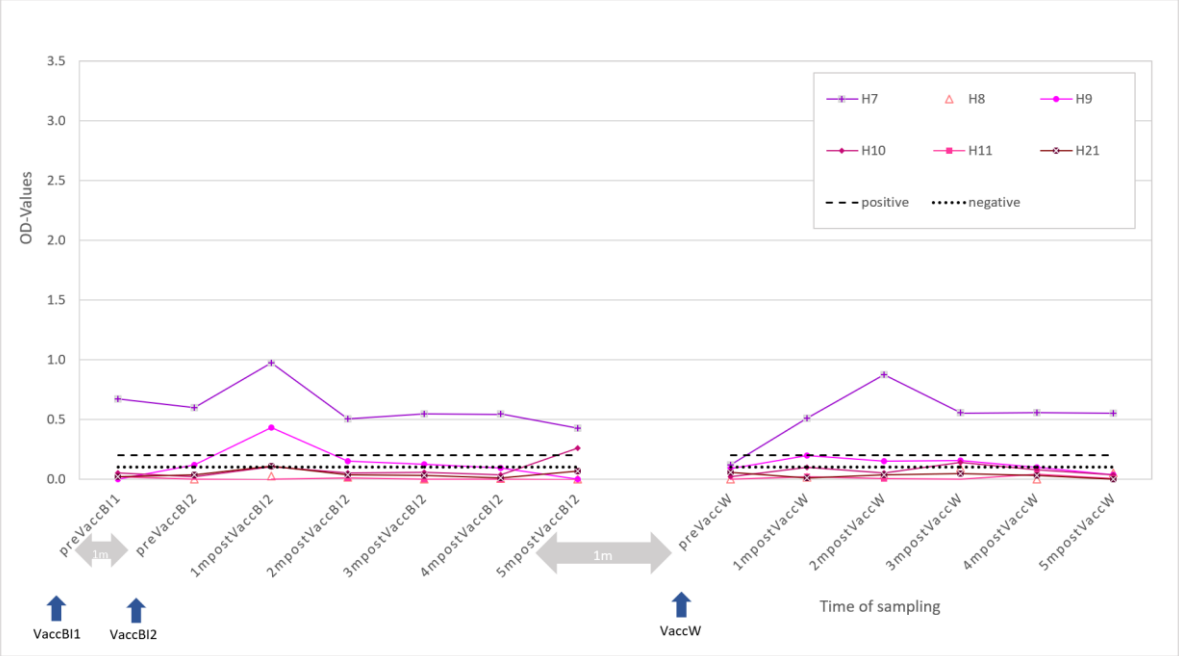


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.4.6 Group C – no vaccination history, vaccinated with BI followed by a booster 6 months later

In Group C 3/6 (50%) horses showed a serological response to summer vaccination. Only one of these horses showed a consistent high EHV-1 antibody titer (OD-Value > 0.2), this horse had a positive EHV-1 pre-study status and was the only animal which showed side effects to the vaccination. That same horse also showed a serological response after the second vaccination, while none of the other horses showed any response (Fig. 10).

Figure 10: Longitudinal EHV-1 antibody concentrations of horses in Group C (no pre-study vacinations) which received a two-step basic immunization in summer followed by a booster in winter (n = 6)

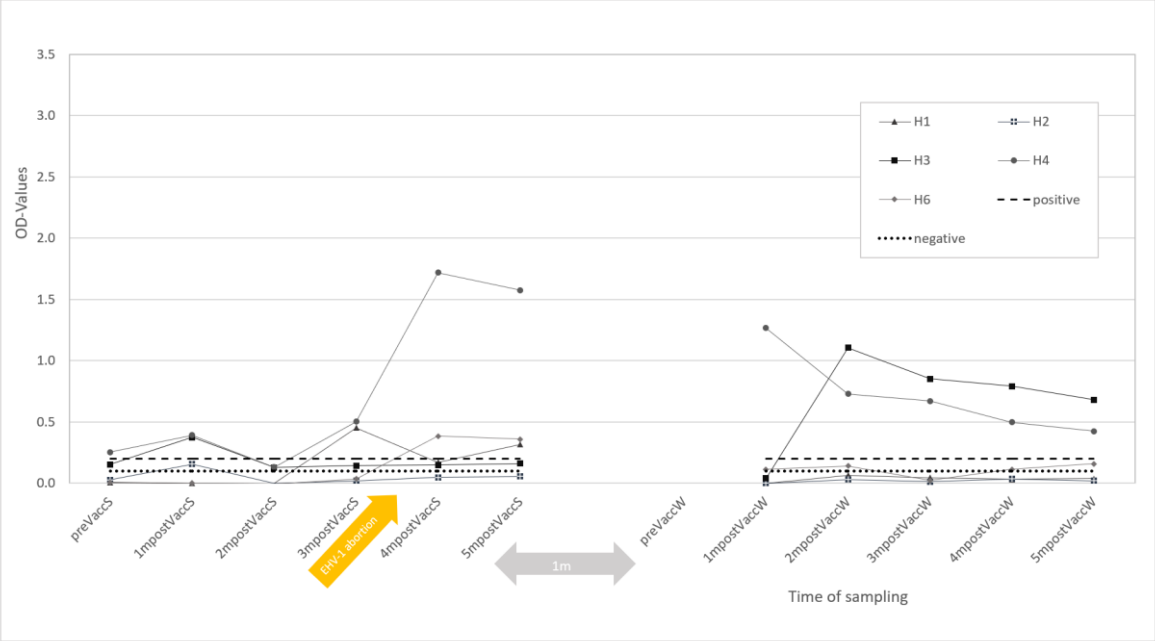


H: Horse number; VaccBI1: first dose of basic immunization; VaccBI2: second dose of the basic immunization; VaccW: winter vaccination; preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; mpostVaccBI2: months after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.4.7 Group D – No vaccination history, natural exposure

EHV-1 abortion occurred in the barn of Group D during the last third of the Summer sampling period (between sampling timepoint 3mS and 4mS). Two of five (40%) horses had EHV-1 antibodies at the time point before EHV-1 abortion occurred, 1/5 (20%) had a non-interpretable result. Only 1/5 horses in the barn, a horse that already had antibodies before the outbreak, showed a strong serological response after the EHV-1 abortion which lasted for at least 7 months. All other horses remained negative or had antibody concentrations similar to before the abortion. Interestingly one horse showed a sudden spike in antibodies with a slow decline afterwards in the second sampling period, unrelated to known natural exposure or vaccination (Fig. 11).

Figure 11: Longitudinal EHV-1 antibody concentrations of horses in control Group D (no pre-study vaccinations; n = 5) which received no vaccinations during the study but may have been exposed to virus by an EHV-1 abortion in the same stable.



H: Horse number; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS;
OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

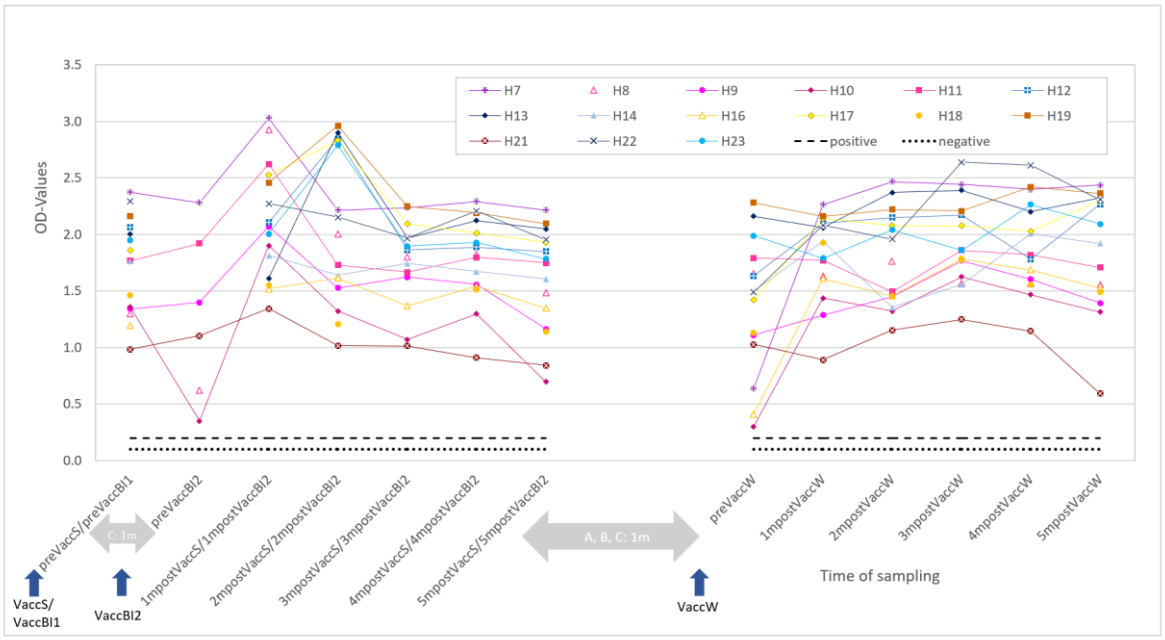
3.1.5 Serological EHV-4 response after vaccination

All horses had a positive EHV-4 pre-study status and all were seropositive following vaccination at all time points. There were no non interpretable results.

3.1.5.1 Serological results of single animals

In every vaccination group, a distinct EHV-4 antibody rise was seen two months after summer vaccination (2mpostVaccS; A, B) or a month after the second BI (1mpostVaccBI2; Group C). After summer vaccination 10/15 horses (67%) showed a serological response, after winter vaccination 7/15 (47%) horses showed a response (Fig. 12).

Figure 12: Longitudinal EHV-4 antibody concentrations in horses vaccinated against EHV-1/4 with different protocols (n = 15)



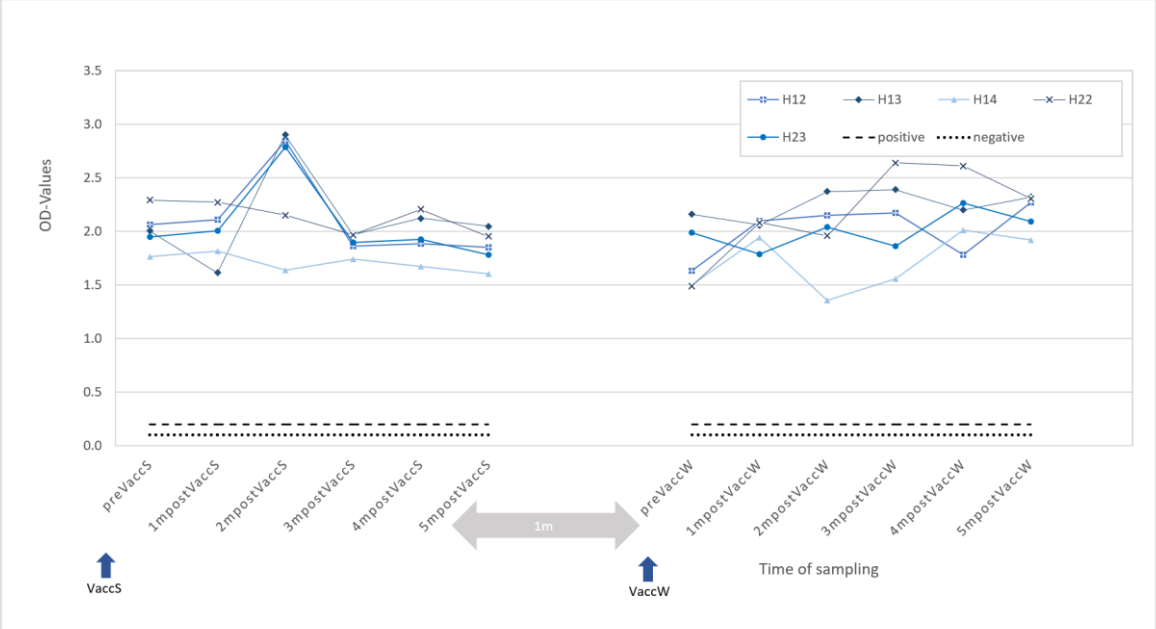
H: Horse number; BI: Basic immunization consisting of two vaccine doses administered 4 weeks apart (VaccBI1, VaccBI2); VaccS: summer vaccination; VaccW: winter vaccination, preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination, preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only); preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only), mpostVaccBI2: months after the second dose of the basic immunization (Group C only); OD: Optical density
Shades of blue: Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study; Shades of yellow: Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI; Shades of purple: Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

In the following figures (Fig 13-16) individual serological responses to EHV-4 are shown based on vaccination group.

3.1.5.2 Group A – Basic immunization > 4 years ago with boosters every 6 months

In Group A 3/5 (60%) showed a peak two months after the summer vaccination, a similar peak was not observed after the winter vaccination. The serological results after the winter vaccination appear arbitrary and not associated with the vaccination (Fig. 13).

Figure 13: Longitudinal EHV-4 antibody concentrations of horses in Group A (regular pre-study vaccinations) which received a summer and winter vaccination during the study (n = 5)

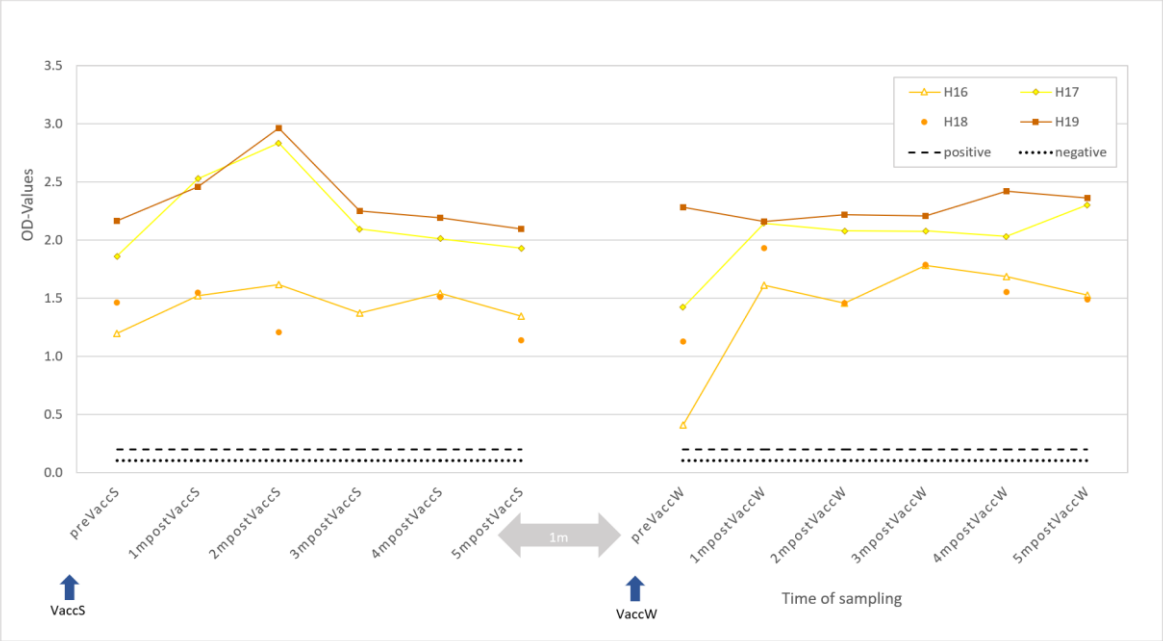


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.5.3 Group B – no vaccination history, vaccinated twice 6 months apart

In Group B 2/4 (50%) horses showed a peak in EHV-4 antibodies 2 months after summer vaccination. After winter vaccination, 3/4 horses displayed a strong antibody rise (Fig. 14).

Figure 14: Longitudinal EHV-4 antibody concentrations of horses in Group B (no pre-study vaccinations) which received a summer and winter vaccination during the study (n = 4)



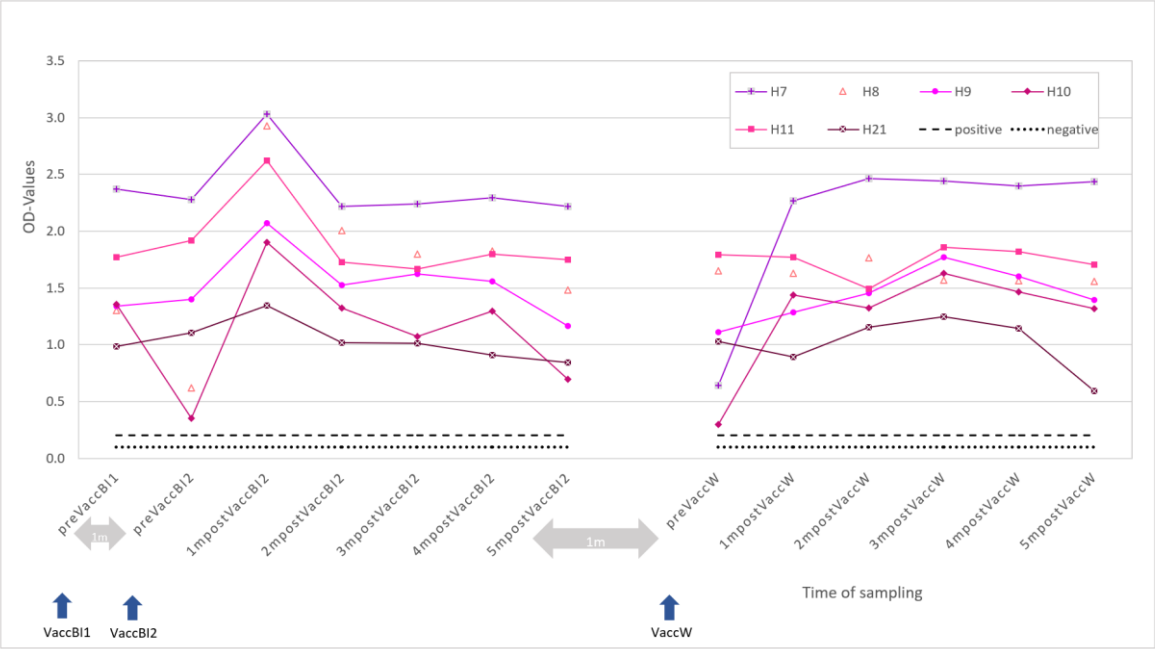
H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density

Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.5.4 Group C – no vaccination history, vaccinated with BI followed by a booster 6 months later

In Group C 5/6 (83%) horses showed a peak in the EHV-4 antibodies 1 month after the second part of the basic immunization (VaccBI2). In the winter sampling period, only 3 horses displayed an increase in EHV-4 antibodies 1 month after the vaccination. One of these horses (H7) had adverse effects to the first part of the BI (Fig. 15).

Figure 15: Longitudinal EHV-4 antibody concentrations of horses in Group C (no pre-study vacinations) which received a two-step basic immunization in summer followed by a booster in winter (n = 6)

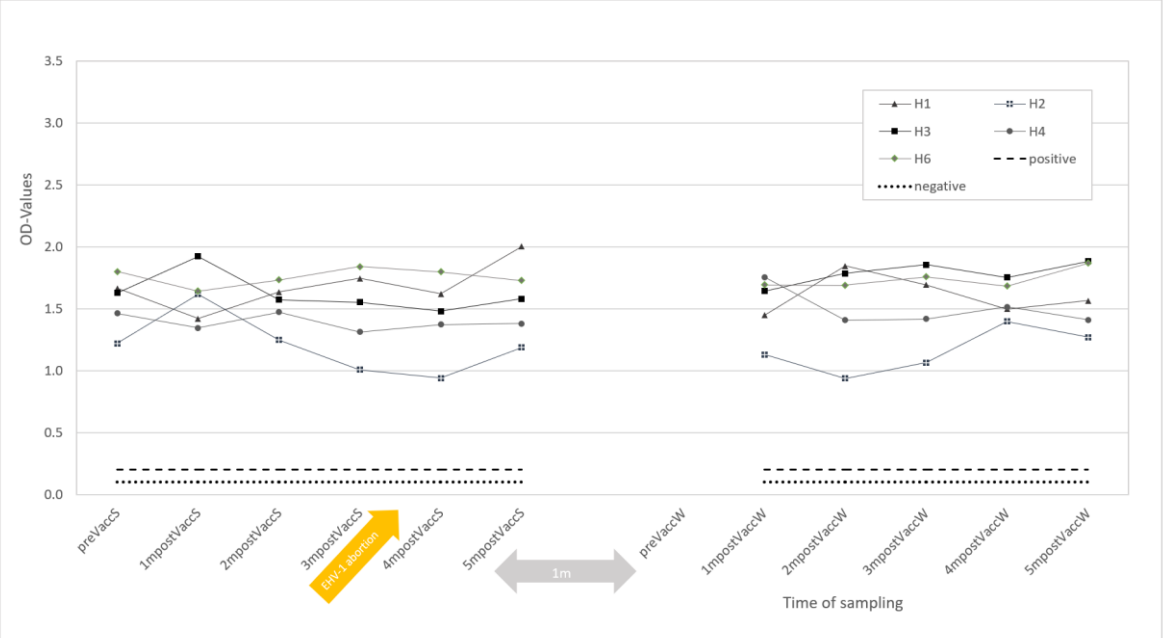


H: Horse number; VaccBI1: first dose of basic immunization; VaccBI2: second dose of the basic immunization; VaccW: winter vaccination; preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; mpostVaccBI2: months after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.4.5 Group D – No vaccination history, natural exposure

The non-vaccinated horses were seropositive for EHV-4 antibodies over the whole sampling period. (Fig. 16). The concentration of EHV-4 antibody titer of the control horses is individual and consistent. A reaction to the EHV-1 abortion in the barn is not seen.

Figure 16: Longitudinal EHV-4 antibody concentrations of horses in control Group D (no pre-study vaccinations; n = 5) which received no vaccinations during the study but may have been exposed to virus by an EHV-1 abortion in the same stable.



Horse number; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS;
OD: Optical density
Values between negative line (…….) and positive line (------) are defined as not interpretable results.

3.1.6 Viral shedding at pre-study status

Before the first vaccination 15 horses had samples taken. The samples from the 5 control horses were taken at a later timepoint without vaccination. Six swabs from Group A-C and 5 from group D were available for analysis. In Group D none of the samples taken at the time points set as ‘before vaccination’ were positive. From the vaccinated animals 4/6 nasal swabs (67%), taken immediately before the first vaccine was administered (VaccS and VaccBI1), were positive for EHV-1 and 5/6 (83%) for EHV-4. All five nasal swabs (100%) taken immediately before the second step of the BI in Group C were positive for EHV-1 and EHV-4. Nine out of fifteen nasal swabs (60%) taken before the second vaccine (VaccW) were positive for EHV-1 and 10/15 (67%) for EHV-4.

3.1.6.1 Contamination of pre-vaccination samples by vaccine vials

As the high prevalence of viral shedding before vaccination seemed unusual and unlikely, contamination of the samples during the sampling procedure was suspected and an experiment conducted to test this hypothesis. Vials as well as gloved hands were sampled after handling the vaccine vial (see Material and Methods).

The swab taken from the outside of the Duvaxyn vial was positive for EHV-1 and EHV-4. One of three swabs (33%) taken from the hands after handling the vaccine vial was positive for EHV-4 but all were negative for EHV-1. In contrast, none of the samples taken from the Tetanus vaccine vial and hands after handling the vaccine bottle were positive for EHV-1 and EHV-4 (Tab. 20). Based on the results of this trial, contamination of the samples taken directly before vaccination was suspected as the syringes with the vaccines had been prepared prior to taking the pre-vaccination nasal swabs.

These timepoints are therefore not presented in the figures and excluded from discussion regarding viral shedding.

Table 20: EHV-1 and EHV-4 DNA contamination of the Duvaxyn vaccine bottle and hands after handling the vaccine bottle

Vaccine	Timepoint	EHV-1 (CT-Value)	EHV-4 (CT-Value)
Duvaxyn	Hands before starting the experiment	neg	neg
	Swab from the outside of a vaccine vial	34.5	31.7
	Hands, after drawing vaccine into the syringe	neg	36.9
	Hands, after changing the needle	neg	neg
	Hands, after removing air from the syringe	neg	neg
	Vaccine eNAT swab	18.5	16.8
	Vaccine UTM swab	18.8	17.1
Tetanus (negative control)	Hands before starting the experiment	neg	neg
	Swab from the outside of a vaccine vial	neg	neg
	Hands, after drawing vaccine into the syringe	neg	neg
	Hands, after changing the needle	neg	neg
	Hands, after removing air from the syringe	neg	neg
	Vaccine eNAT swab	neg	neg
	Vaccine UTM swab	neg	neg

CT: Cycle Threshold; neg: negative; eNAT: nasal swab containing a guanidine-thiocyanate based medium; UTM: nasal swab containing a Universal Transport Medium

3.1.7 EHV-1 viral shedding after vaccination

3.1.7.1 Viral shedding results of sample groups

Of the vaccinated horses 8/15 (53%) were positive for EHV-1 at one timepoint after vaccination.

None of the unvaccinated horses were positive for EHV-1 before the EHV-1 abortion in the barn occurred, 2/5 (40%) of the horses (H1, H4) were positive for EHV-1 three months after the outbreak. One of these horses showed a sustained peak in EHV-1 antibody concentrations after the outbreak (see Fig. 10), the other horse did not show an antibody response.

EHV-1 PCR results based on vaccination group at each time point are shown in Table 21.

Table 21: EHV-1 viral shedding in 21 horses vaccinated against EHV-1/4 with three different protocols or exposed to EHV-1 at various timepoints

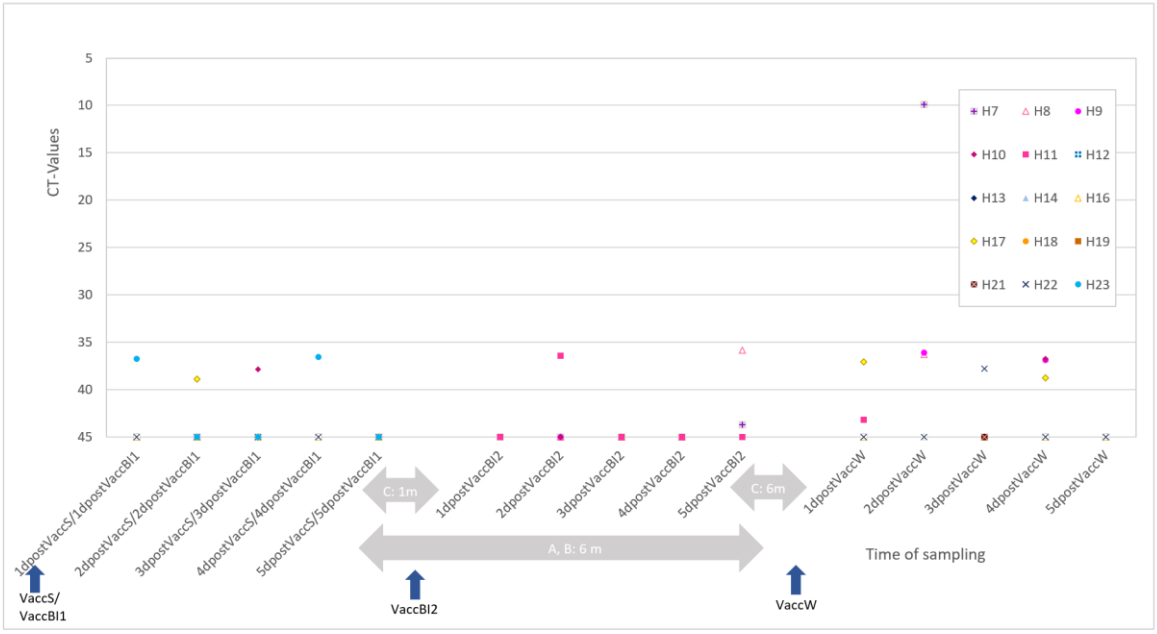
EHV-1 **Timepoint of sampling**	**A (n = 5)**	**B (n = 4)**	**C (n = 6)**	**D (n = 5)**	**Total A-C (n = 15)**
preVaccS/preVaccBI1	*50% (1/2)*	*67% (2/3)*	*100% (1/1)*	*0% (0/5)*	*67% (4/6)*
1dpostVaccS/1dpostVaccBI1	50% (1/2)	0% (0/3)	0% (0/5)	0% (0/5)	10% (1/10)
2dpostVaccS/2dpostVaccBI1	0% (0/2)	33% (1/3)	0% (0/5)	0% (0/5)	10% (1/10)
3dpostVaccS/3dpostVaccBI1	0% (0/2)	0% (0/4)	25% (1/4)	0% (0/5)	10% (1/10)
4dpostVaccS/4dpostVaccBI1	0% (0/2)	0% (0/4)	0% (0/4)	0% (0/5)	0% (0/10)
5dpostVaccS/5dpostVaccBI1	0% (0/2)	0% (0/4)	0% (0/4)	0% (0/5)	0% (0/10)
preVaccBI2			*100% (5/5)*		*100% (5/5)*
1dpostVaccBI2			0% (0/4)		0% (0/4)
2dpostVaccBI2			20% (1/5)		20% (1/5)
3dpostVaccBI2			0% (0/5)		0% (0/5)
4dpostVaccBI2			0% (0/5)		0% (0/5)
5dpostVaccBI2			40% (2/5)		40% (2/5)
preVaccW	*40% (2/5)*	*60% (3/4)*	*67% (4/6)*	*20% (1/5)*	*60% (9/15)*
1dpostVaccW	0% (0/5)	33% (1/3)	0% (0/6)	0% (0/5)	7% (1/14)
2dpostVaccW	0% (0/5)	0% (0/4)	50% (3/6)	20% (1/5)	20% (3/15)
3dpostVaccW	0% (0/5)	0% (0/4)	0% (0/6)	20% (1/5)	0% (0/15)
4dpostVaccW	0% (0/5)	25% (1/4)	33% (2/6)	0% (0/5)	20% (3/15)
5dpostVaccW	0% (0/5)	0% (0/3)	0% (0/6)		0% (0/14)

preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination;
preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only); preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only); dpostVaccBI1: days after the first dose of the basic immunization (Group C only);
dpostVaccBI2: days after the second dose of the basic immunization (Group C only);
BI: Basic immunization consisting of two doses given 4 weeks apart;
Group A: BI >4 years ago, q6months booster which continued throughout the study
Group B: No vaccination history, vaccinated two times, six months apart without prior BI
Group C: No vaccination history, BI in summer followed by a booster after six months
Group D: No vaccination history and no vaccination as part of the study, natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period
Timepoints before each vaccination administration are written in italics

3.1.7.2 Viral shedding results of single animals

Results of nasal shedding from individual horses based on vaccination group before and after vaccination are shown in Figure 16.

Figure 17: EHV-1 nasal shedding over 5 days in horses vaccinated against EHV-1/4 with different protocols (n = 15)



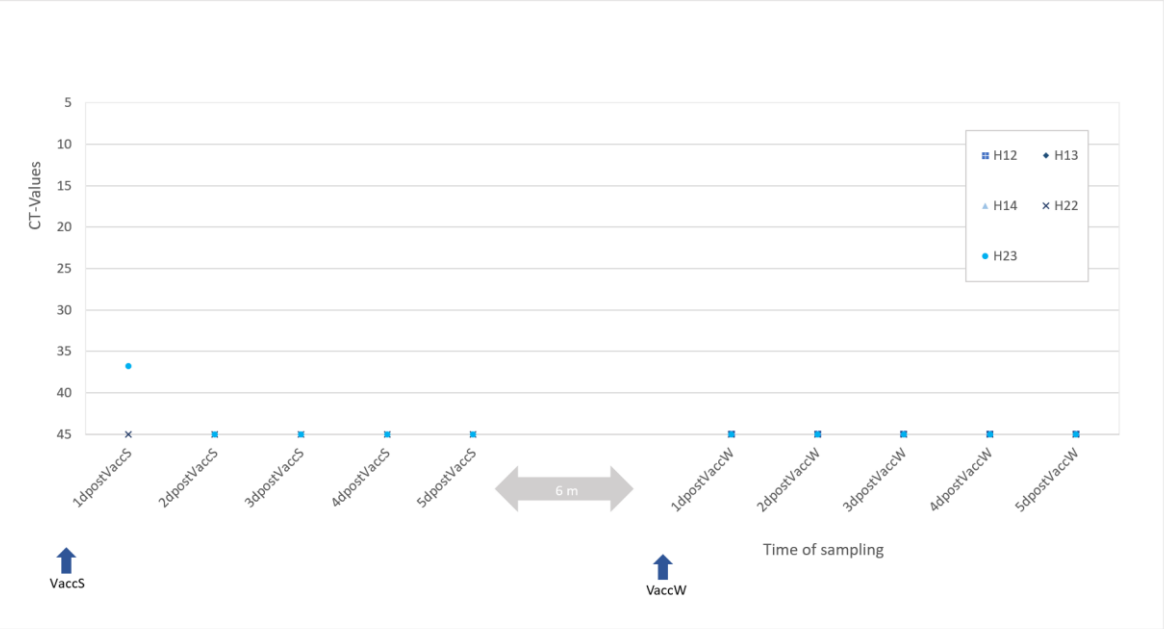
H: Horse number; BI: Basic immunization consisting of two vaccine doses administered 4 weeks apart (VaccBI1, VaccBI2); VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; dpostVaccBI1: days after the first dose of the basic immunization (Group C only); dpostVaccBI2: days after the second dose of the basic immunization (Group C only); CT: Cycle Threshold
Shades of blue: Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study; Shades of yellow: Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI; Shades of purple: Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

In the following figures (Fig 18 - 21) nasal shedding of EHV-1 of every horse is shown separated by vaccination group.

3.1.7.3 Group A – Basic immunization > 4 years ago with boosters every 6 months

In Group A, only one horse was positive for EHV-1 DNA in the nasal swab one day after vaccination (Fig. 18).

Figure 18: Longitudinal EHV-1 nasal shedding of horses in Group A (regular pre-study vaccinations) which received a summer and winter vaccination during the study (n = 5)

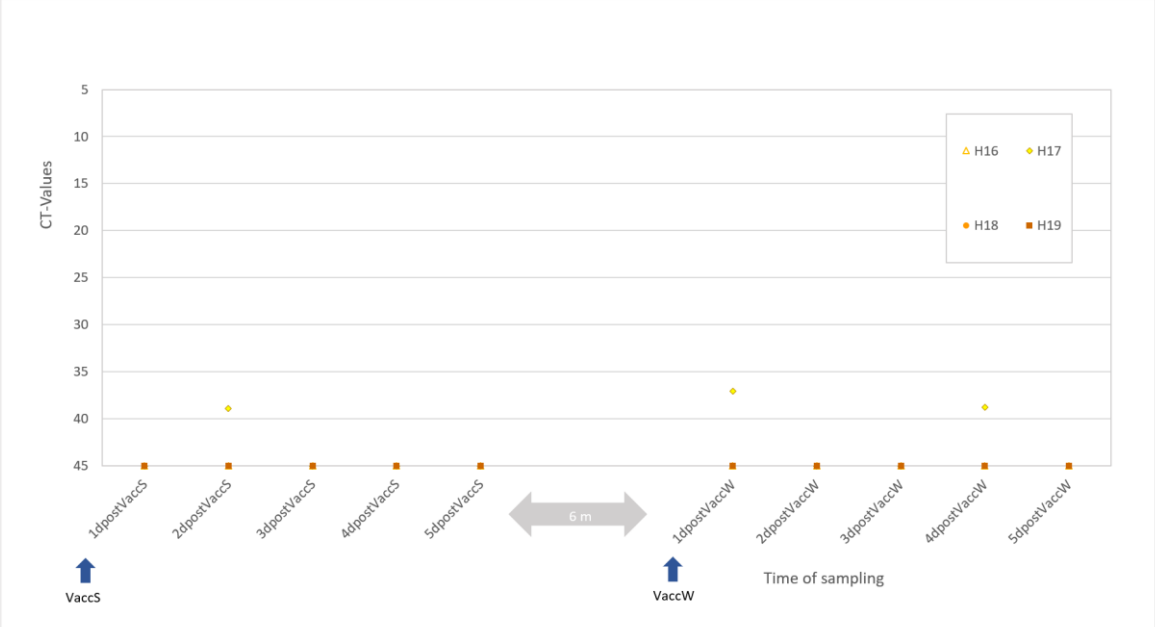


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.7.3 Group B – no vaccination history, vaccinated twice 6 months apart

In Group B, one horse was EHV-1 positive at three timepoints during the whole sampling period. This horse belonged to one of the two of this Group, that displayed a consistent high EHV-1 antibody titer over the whole sampling period (Fig. 19).

Figure 19: Longitudinal EHV-1 nasal shedding of horses in Group B (no pre-study vaccinations) which received a summer and winter vaccination during the study (n = 4)

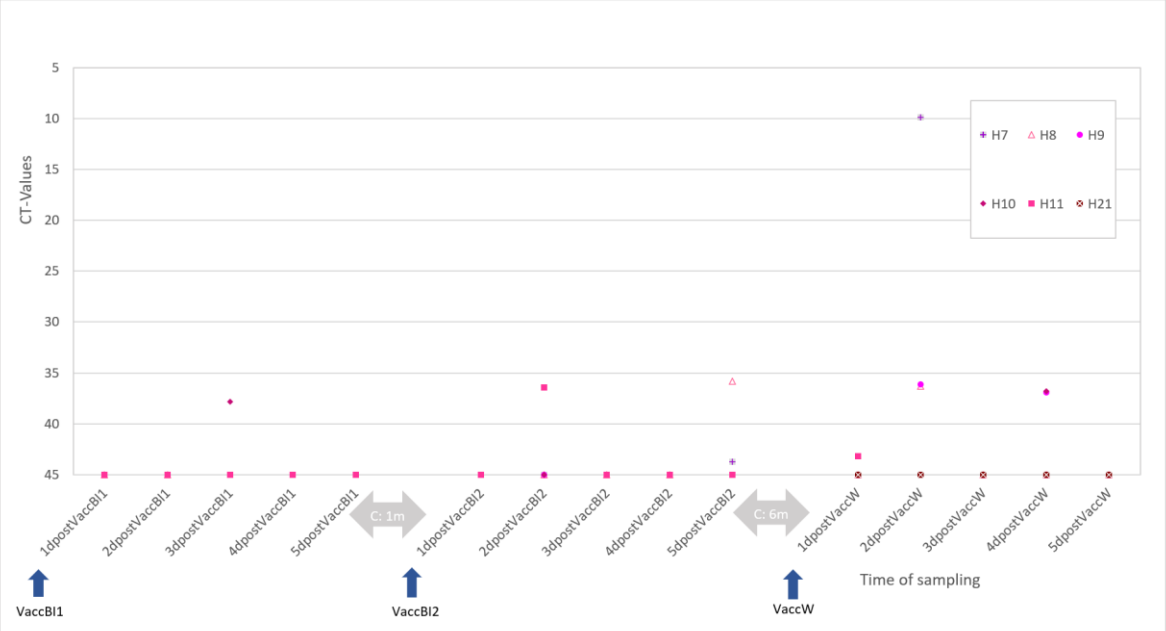


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.7.5 Group C – no vaccination history, vaccinated with BI followed by a booster 6 months later

In Group C one horse was shedding EVH-1 after the first part of BI. After the second part of the BI, three horses were EHV-1 positive. One horse had a high CT-value of 10 at one time point, this was the same horse that showed adverse effects after administration of the first part of the BI. This horse also showed adequate EHV-1 antibody titers over the whole sampling period (Fig. 20).

Figure 20: Longitudinal EHV-1 nasal shedding of horses in Group C (no pre-study vacinations) which received a two-step basic immunization in summer followed by a booster in winter (n = 6)



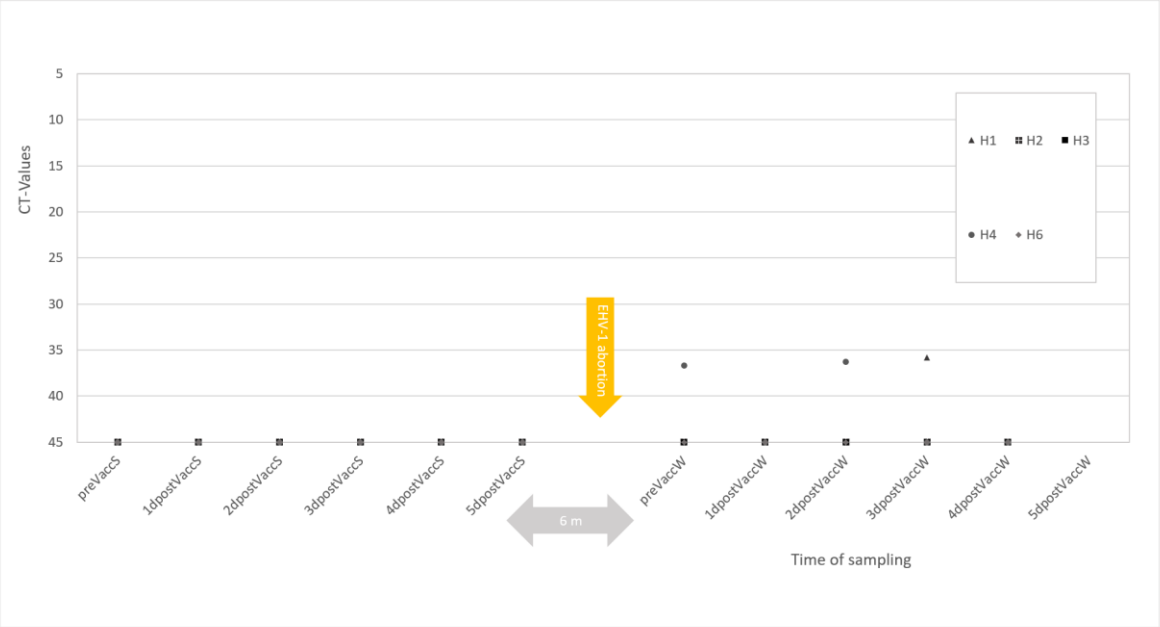
H: Horse number; VaccBI1: first dose of basic immunization; VaccBI2: second dose of the basic immunization; VaccW: winter vaccination ; dpostVaccBI1: days after the first dose of the basic immunization; dpostVaccBI2: days after the second dose of the basic immunization; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.7.6 Group D – No vaccination history, natural exposure

Before natural EHV-1 infection in the control Group occurred, none of the horses were shedding EHV-1 virus whereas 14 weeks after EHV-1 abortion in the barn, two horses (H1, H4) were shedding EHV-1 at three timepoints (Fig. 21).

The horse that was shedding EHV-1 at two timepoints (H4) was EHV-1 seropositive during the whole Winter sampling period (Fig. 15). The other EHV-1 positive horse (H1) was seronegative in the Winter sampling period.

Figure 21: Longitudinal EHV-1 nasal shedding of horses in control Group D (no pre-study vaccinations; n = 5) which received no vaccinations during the study but may have been exposed to virus by an EHV-1 abortion in the same stable.



H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.8 EHV-4 viral shedding after vaccination

3.1.8.1 Viral shedding results of sample groups

Ten of fifteen (67%) horses were positive for EHV-4 at one timepoint after vaccination (summer or winter or BI1/BI2). One of six (17%) horses of the control Group was positive for EHV-4 (H3) at least at one timepoint before natural exposure due to an EHV-1 abortion in the barn occurred. After that timepoint, none of the horses of these group were positive for EHV-4. EHV-4 results based on vaccination group at each time point are shown in Table 22.

Table 22: EHV-4 viral shedding in 20 horses vaccinated against EHV-1/4 with three different protocols or sampled as control horses

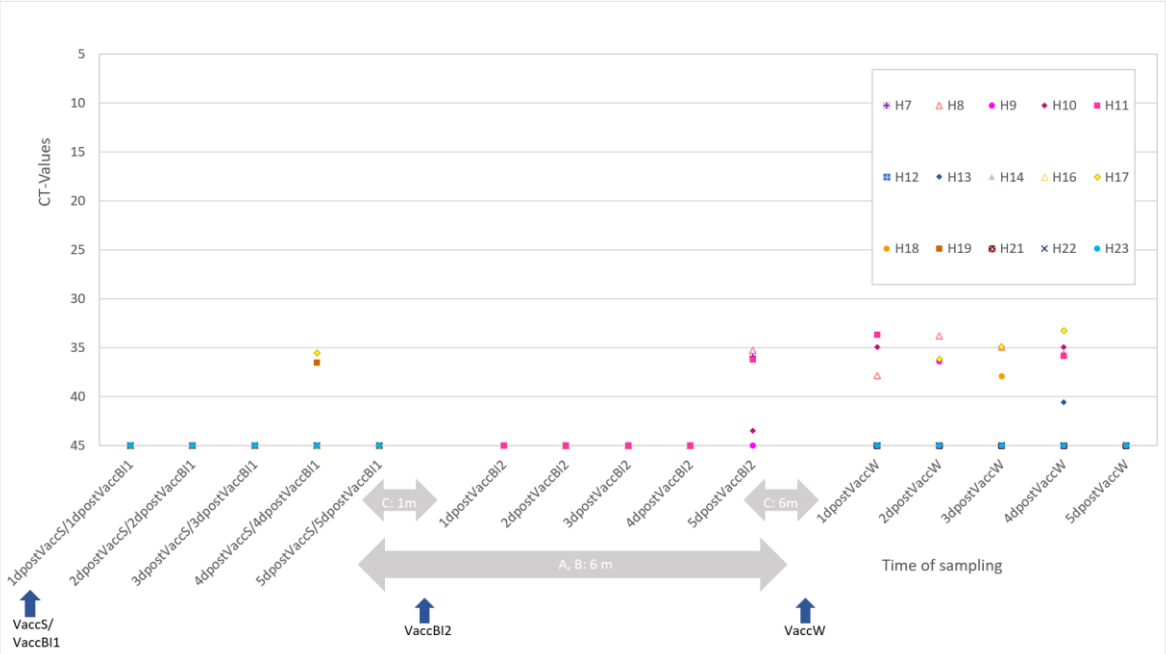
EHV-4 **Timepoint of sampling**	**A (n = 5)**	**B (n = 4)**	**C (n = 6)**	**D (n = 5)**	**Total A-C (n = 15)**
preVaccS/preVaccBI1	*50% (1/2)*	*100% (3/3)*	*100% (1/1)*	*0% (0/5)*	*83% (5/6)*
1dpostVaccS/1dpostVaccBI1	0% (0/2)	0% (0/3)	0% (0/5)	0% (0/5)	0% (0/10)
2dpostVaccS/2dpostVaccBI1	0% (0/2)	0% (0/3)	0% (0/5)	20% (1/5)	0% (0/10)
3dpostVaccS/3dpostVaccBI1	0% (0/2)	0% (0/4)	0% (0/4)	0% (0/5)	0% (0/10)
4dpostVaccS/4dpostVaccBI1	0% (0/2)	50% (2/4)	0% (0/4)	0% (0/5)	20% (2/10)
5dpostVaccS/5dpostVaccBI1	0% (0/2)	0% (0/4)	0% (0/4)	0% (0/5)	0% (0/10)
preVaccBI2			*100% (5/5)*		*100% (5/5)*
1dpostVaccBI2			0% (0/4)		0% (0/4)
2dpostVaccBI2			0% (0/5)		0% (0/5)
3dpostVaccBI2			0% (0/5)		0% (0/5)
4dpostVaccBI2			0% (0/5)		0% (0/5)
5dpostVaccBI2			60% (3/5)		60% (3/5)
preVaccW	*60% (3/5)*	*75% (3/4)*	*67% (4/6)*	*0% (0/5)*	*67% (10/15)*
1dpostVaccW	0% (0/5)	0% (0/3)	50% (3/6)	0% (0/5)	21% (3/14)
2dpostVaccW	0% (0/5)	25% (1/4)	33% (2/6)	0% (0/5)	20% (3/15)
3dpostVaccW	0% (0/5)	50% (2/4)	17% (1/6)	0% (0/5)	20% (3/15)
4dpostVaccW	20% (1/5)	25% (1/4)	50% (3/6)	0% (0/5)	33% (5/15)
5dpostVaccW	0% (0/5)	0% (0/3)	0% (0/6)		0% (0/14)

preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination;
preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only); preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only); dpostVaccBI1: days after the first dose of the basic immunization (Group C only);
dpostVaccBI2: days after the second dose of the basic immunization (Group C only);
BI: Basic immunization consisting of two doses given 4 weeks apart;
Group A: BI >4 years ago, q6months booster which continued throughout the study
Group B: No vaccination history, vaccinated two times, six months apart without prior BI
Group C: No vaccination history, BI in summer followed by a booster after six months
Group D: No vaccination history and no vaccination as part of the study, natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period
Timepoints before each vaccination administration are written in italics

3.1.8.2 Viral shedding results of single animals

Overall, EHV-4 shedding after vaccination occurred occasionally. In every vaccination group, EHV-4 positive results were detected more frequent after winter vaccination compared to summer vaccination or BI immunization. After summer vaccination, 2/15 (13%) horses were shedding EHV-4 whereas 7/15 (47%) horses were EHV-4 positive after winter vaccination (Fig. 21). Results of nasal shedding from individual horses before and after vaccination of every vaccination group are shown in Figure 22.

Figure 22: Longitudinal EHV-4 nasal shedding in horses vaccinated against EHV-1/4 with different protocols (n = 15)



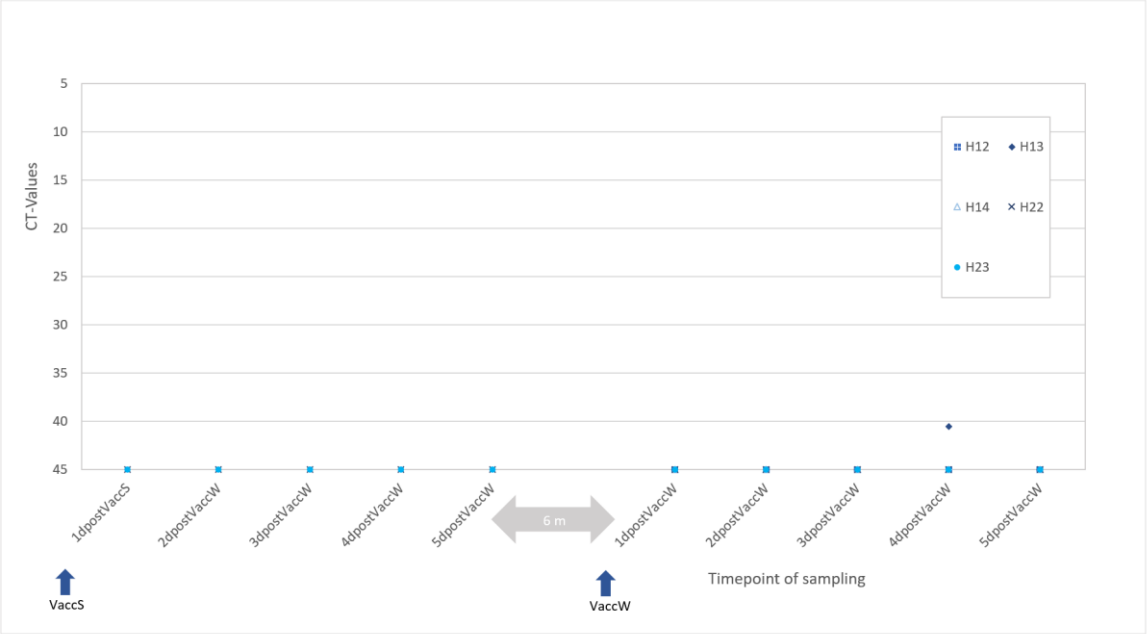
H: Horse number; BI: Basic immunization consisting of two vaccine doses administered 4 weeks apart (VaccBI1, VaccBI2); VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; dpostVaccBI1: days after the first dose of the basic immunization (Group C only); dpostVaccBI2: days after the second dose of the basic immunization (Group C only); CT: Cycle Threshold
Shades of blue: Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study; Shades of yellow: Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI; Shades of purple: Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

In the following figures (Fig. 23-26) nasal shedding of EHV-4 of every horse is shown based on vaccination group.

3.1.8.3 Group A – Basic immunization > 4 years ago with boosters every 6 months

In Group A, only one horse was EHV-4 positive in the nasal swab four days after winter vaccination (Fig. 23).

Figure 23: Longitudinal EHV-4 nasal shedding of horses in group A (regular pre-study vaccinations) which received a summer and winter vaccination during the study (n = 5)

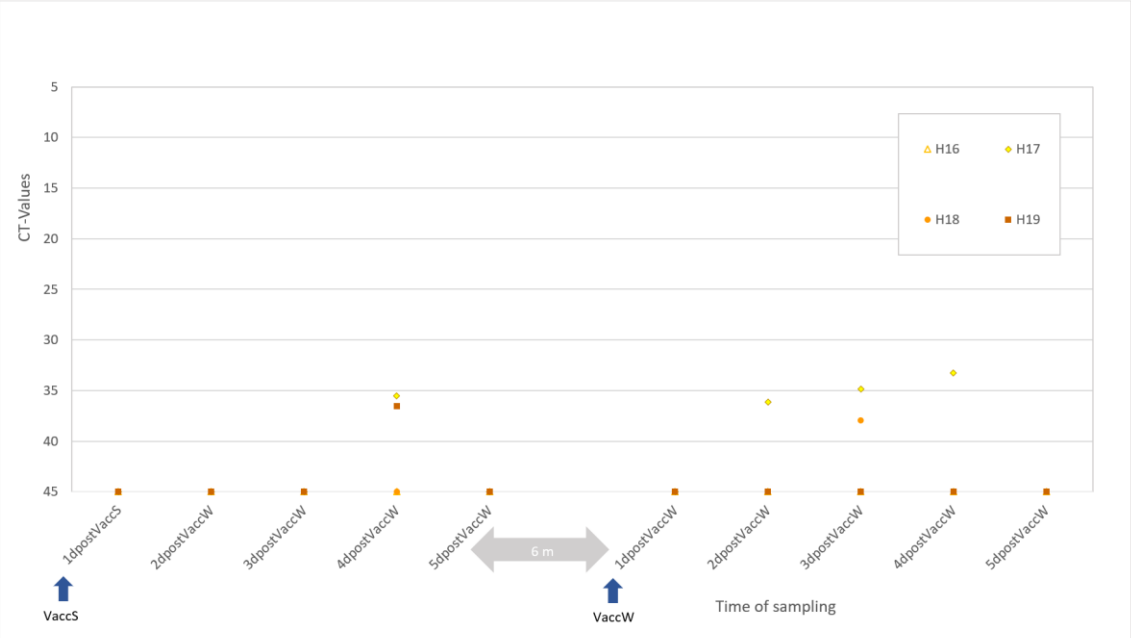


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.8.4 Group B – no vaccination history, vaccinated twice 6 months apart

In Group B, 3/4 (75%) horses were positive for EHV-4 at four different timepoints after summer and winter vaccination. One horse was shedding EHV-4 consistently three days in a row after winter vaccination (Fig. 24).

Figure 24: Longitudinal EHV-4 nasal shedding of horses in Group B (no pre-study vaccinations) which received a summer and winter vaccination during the study (n = 4)

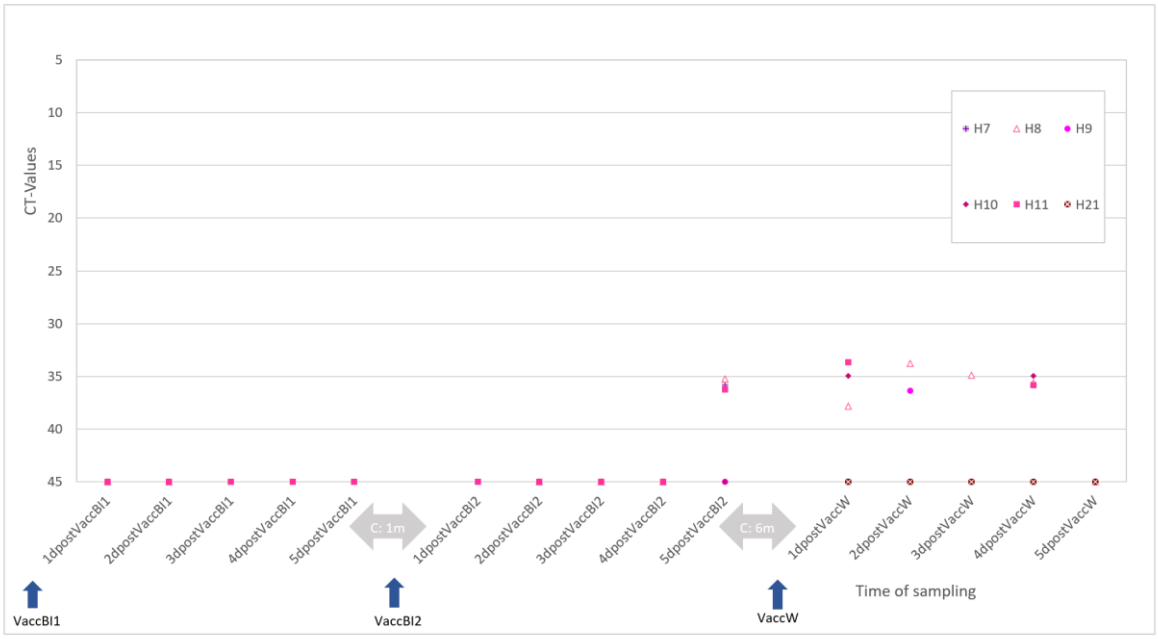


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.8.5 Group C – no vaccination history, vaccinated with BI followed by a booster 6 months later

Five of six horses of this group (83%) were EHV-4 positive at least at one sampling timepoint. EHV-4 viral shedding increased after administration of every vaccine during the sampling period: None of the horses was positive after BI1 whereas 3/6 (50%) of the horses (H7, H8, H11) were shedding EHV-4 5 days after administration of BI2. After winter vaccination, 4/6 (67%) of the horses were positive for EHV-4 in the nasal swab. One of the horses was shedding EHV-4 daily from the first to the fourth day after winter vaccination (Fig. 25).

Figure 25: Longitudinal EHV-4 nasal shedding of horses in Group C (no pre-study vacinations) which received a two-step basic immunization in summer followed by a booster in winter (n = 6)

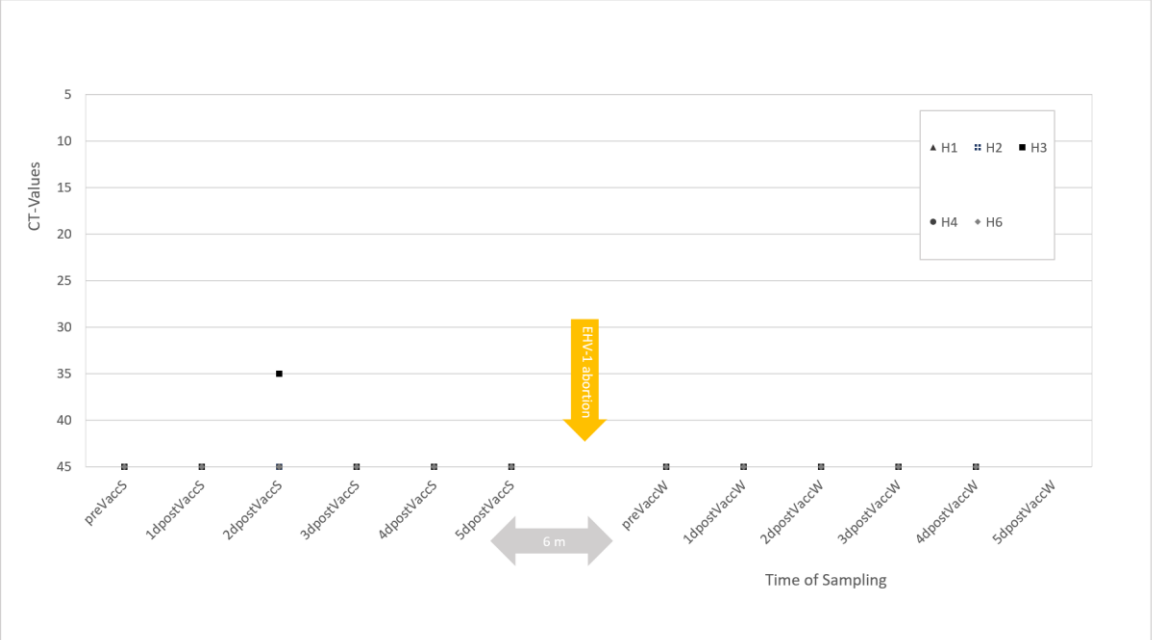


H: Horse number; VaccBI1: first dose of basic immunization; VaccBI2: second dose of the basic immunization; VaccW: winter vaccination ; dpostVaccBI1: days after the first dose of the basic immunization; dpostVaccBI2: days after the second dose of the basic immunization; dpostVaccW: days post winter vaccination; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.1.8.6 Group D – No vaccination history, natural exposure

In the control Group, one horse (H3) was shedding EHV-4 positive at one summer sampling timepoint (Fig. 26).

Figure 26: Longitudinal EHV-4 nasal shedding of horses in control Group D (no pre-study vaccinations; n = 5) which received no vaccinations during the study but may have been exposed to virus by an EHV-1 abortion in the same stable.

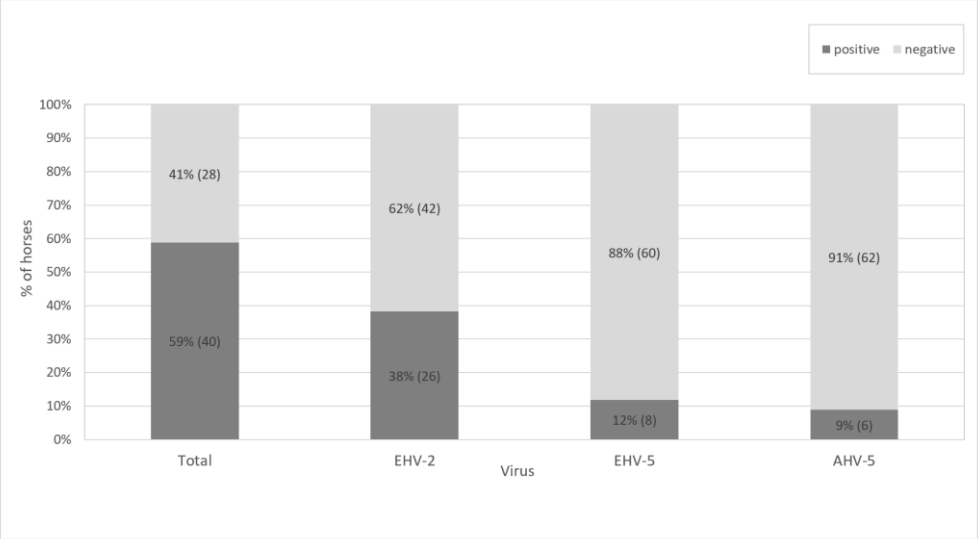


H: Horse number; VaccS: summer vaccination; VaccW: winter vaccination; dpostVaccS: days post summer vaccination; dpostVaccW: days post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period; CT: Cycle Threshold
The cut-off for a positive result is CT 45, all negative results are presented on the baseline of the x-axis, all positive results include CT-Values < 45. Note overlapping of symbols of individual horses on the x-axis.

3.2 Part B – Screening for equine gamma herpesviruses in healthy horses

In this study, 59% (40/68) of horses were positive for equine gammaherpesviruses; 38% (26/68) were positive for EHV-2, 12% (8/68) for EHV-5 and 9% (6/68) for AHV-5 (Fig. 27).

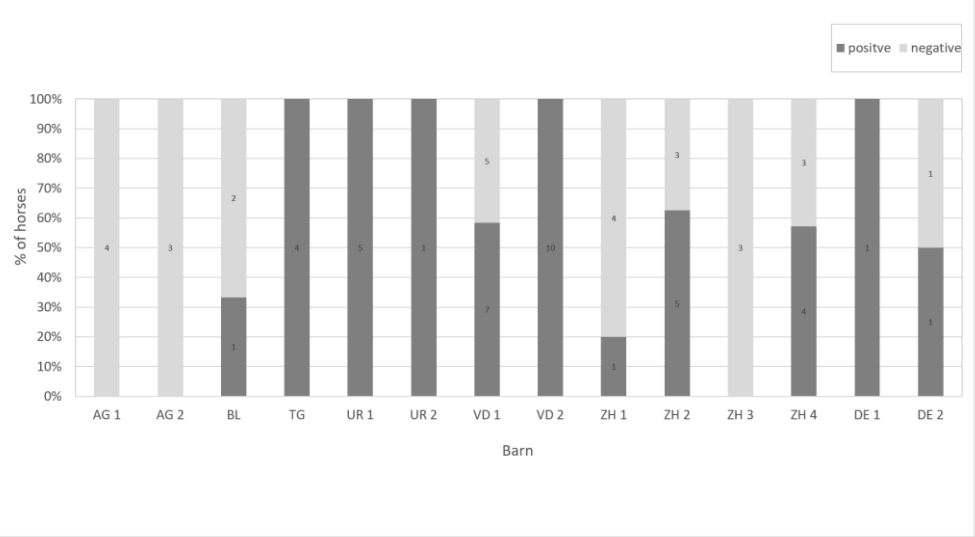
Figure 27: Prevalence of different equine gammaherpesviruses in the tested horses in Switzerland
(n = 68)



Absolute numbers of horses are indicated within the column in parentheses

An overview of positive results based on region of origin is shown in Figure 28. In 11/14 barns, equine gammaherpesviruses were detected. In five barns, every examined horse (TG, UR 1, UR 2, VD2, DE 1) was positive for equine gammaherpesviruses. No positive gammaherpesvirus result was measured in the two barns of canton Aargau and in one barn of canton Zurich.

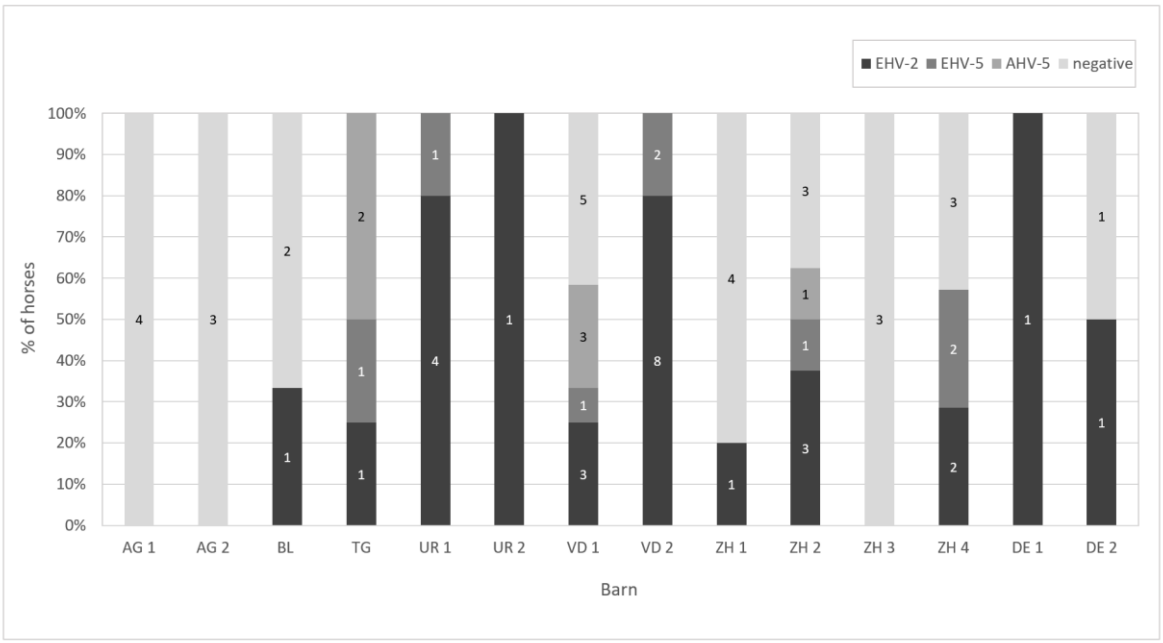
Figure 28: Prevalence of equine gammaherpesviruses in 68 horses from 14 different barns



Absolute numbers of horses are indicated within the columns

Prevalence of each gammaherpesvirus based on barn of origin is shown in Figure 29. EHV-2 was present in every barn where at least one gammaherpesvirus positive result was obtained. EHV-5 was detected in 6/14 (43%) barns and AHV-5 was found in 3/14 (21%) barns.

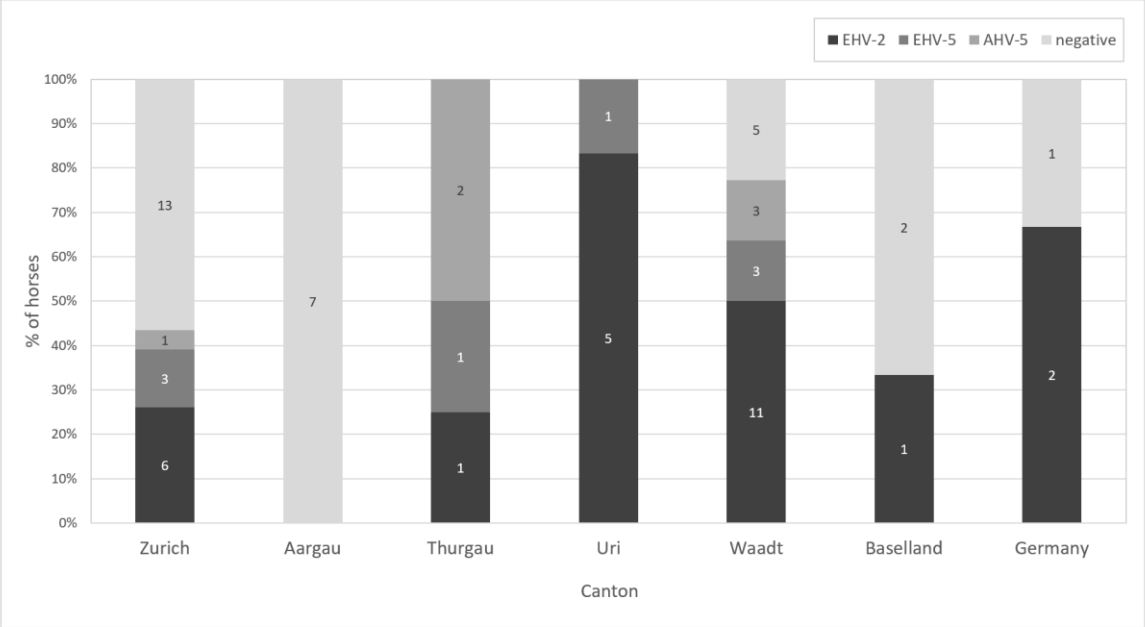
Figure 29: Distribution of different equine gamma herpesviruses isolated from 68 horses from 14 different barns



Absolute numbers of horses are indicated within the column

Prevalence of each gammaherpesvirus based on canton of origin is shown in Figure 30. Aargau was the only canton in which Gamma Herpesviruses were not detected. In Thurgau and Uri all tested horses were positive for at least one gamma herpesvirus.

Figure 30: Prevalence of equine herpesviruses referred to the canton and virus-isolate



Absolute numbers of horses are indicated within the column

4 Discussion

This study showed that 100% of horses included in the study had antibodies against EHV-4 irrespective of vaccination status, while only 40% of regularly vaccinated and 25% of unvaccinated horses had a positive pre-study status. A transient peak in EHV-4 antibodies following summer vaccination and basic immunization was detected, while only a short-lived antibody response was seen in approximately 50% of horses after vaccination against EHV-1. Viral shedding occurred after vaccination in 53% of horses for EHV -1 and 60% for EHV-4. The pre vaccination swab of 80% and 87% of horses was positive for EHV 1 and 4 respectively due to contamination of the nasal swab with DNA from the vaccine bottle from the hands of the researcher. One of the control horses shed EHV-4 at one timepoint.

4.1 PART A – EHV-1 and EHV-4 shedding and antibody response after vaccination

4.1.1 EHV-4 Serology

The high EHV-4 serologic prevalence of 100% in our study population corresponds to previous worldwide investigations[30,32,86,91] with reported seroprevalences between 80% in Turkey[32] to 95 – 100 % in Germany,[91] Australia[86] and New Zealand.[30] In our study every animal, with or without recent vaccination history, showed a positive EHV-4 antibody titer at every timepoint of sampling, even before the first vaccination was administered. EHV-4 is suggested to be a commonly found virus in the equine population that horses are likely exposed to repeatedly and therefore a strong immune response is mounted. This is supported by the results from the analysis of nasal shedding in our study, where one of the horses in the control was shedding EV-4 at one time point, despite never receiving a vaccination.

Compared to the control group, a distinct but short-lived EHV-4 antibody peak in all vaccination groups was detected after administration of the summer vaccine and after the basic immunization. However, besides this peak no distinct influence of the vaccine on EHV-4 antibody curves was detected and the EHV-4 trajectory of the control group is comparable with the vaccination groups. There are few investigations on EHV-4 antibody response to vaccination. Measurements of EHV-4 antibody titers in 20 foals after a two-step immunization with a combined IWVV (Duvaxyn®EHV-1/4) 4 weeks apart and subsequent challenge EHV-4 infection showed induction of EHV-4 antibodies two weeks after first vaccination and which were boosted after the second vaccination. However, post vaccination titers never reached the concentrations induced after virus challenge.[219] A distinct increase in EHV-4 antibody titer after booster vaccination could not be detected in our study population. Although horses which dropped under the pre-study status at the beginning of winter sampling period, showed an increase in EHV-4 antibody titers, this peak was lower compared to the summer sampling period. Further investigations are necessary to determine importance of a booster vaccination.

4.1.2 EHV-1 Serology

Contrary to the high EHV-4 seroprevalence, EHV-1 antibodies were detected only in 17% of horses without a vaccination history at pre-study status. This is similar to other reports were seroprevalence in naïve, adult horses was reported to range from 20%[30] in New Zealand to 52% in Turkey.[32] Seroprevalence of horses that were regularly vaccinated before this study and continued to be vaccinated throughout the study was slightly higher at pre-study status (40%) but does not accomplish expectations of an efficient immune response. With respect to the small study population, no convincing conclusion on beneficial of regular vaccination is possible and further research is needed. Also cellular immunity should also be taken into account, not simply the antibody response when considering the effects of a vaccine. We only investigated the antibody response in this study.

The EHV-1 antibody response to vaccination was different in every horse but generally low. Especially in the group of regularly vaccinated horses, distinct differences in terms of EHV-1 antibody response were apparent. Only 2/5 of these horses displayed consistent high EHV-1 antibody concentrations, whereas the other 3 horses did not show an efficient immune response. These findings correlate with previous investigations[30,32,195]. Seroconversion following vaccination with an inactivated whole virus EHV-1/4 vaccine (DuvaxynTM, Fort Dodge) occurred in less than 30% and 50% of mares and foals, respectively.[133] Overall, our investigation supports the hypothesis that every horse reacts differently but generally moderate to the EHV-1 component of the vaccine and further research is needed.

EHV-1 antibody response to vaccination was moderate in this study. Only horses with pre-existing antibodies (4 out of 15 from groups A-C) remained EHV-1 antibody positive during the whole study and showed clear peaks after vaccination. Of the horses without pre-existing antibodies (n = 11) only three became antibody positive after vaccination: One remained positive over several timepoints, was negative after 6 months but became positive again after the booster (H16, group B). The other two (H9, group C and H23, group A) showed only weak positive results at one timepoint (1 month after summer vaccination) before reverting to a negative status. Horses with previous vaccination history did not display consistently higher EHV-1 antibody concentrations compared to horses not vaccinated before. This moderate antibody response after vaccination is in agreement with previous investigations about longevity of humoral EHV-1 antibodies after infection.[133,192,309] In our study, 8/15 horses seroconverted, defined as at least one positive EHV-1 OD value after vaccination. However, only 5/15 of the vaccinated horses showed consistent high antibody concentrations during the sampling period of six months. This is in agreement with previous investigations, considering that the examined horses of these studies did not have a prior vaccination history. No comparable investigations following our vaccination protocol is available. A vaccination trial with an interval of 60 days did not result in either increased SN-titers or whole EHV-1 ELISA values in regularly vaccinated pregnant mares.[192] In a different vaccine trials, only a minority of vaccinated mares responded with a significant increase in serum antibodies.[133] Despite five repeated vaccinations over a period of 8 months with an interval of 20, 60, 90 and 60 days, antibody and cellular immunity did not always result in increasing immunity.[195] Measurement of serum antibody response to vaccination is commonly used as an indicator of vaccine

efficacy but antibody response alone may not protect against infection. There are case reports of clinical EHV-1 infection with a high titer of neutralizing antibodies.[52,66]

In our study, the response to two different vaccination protocols for horses with no vaccination history were evaluated and compared to a group with regular vaccination. Horses in the group that received BI followed by a booster had a lower seroprevalence than horses vaccinated twice without basic immunization and regularly vaccinated horses. This low seroprevalence however has to be interpreted with caution, as our numbers were low and results from some horses, particularly in Group C, could not be interpreted as they fell into the 'grey zone' of the test. However, non-interpretable results were obtained in the vaccinated groups as frequent as in the control group. Our results are in agreement with another study where no significant increase in SN-titers was detected following administration of two vaccines 28 days apart in unvaccinated horses.[195] The lack of response after the basic immunization followed by the booster is particularly discouraging as 50% and 40% of the horses were shedding EHV-1 after the second step of the BI and the booster, respectively.

In the regularly vaccinated group, a response to vaccination was seen in 3/5 of animals after the first vaccination and 2/5 of horses after the second administration. In one of the animals was the antibody response however moderate and only positive at one sampling timepoint after summer vaccination. There are few investigations on serological response of horses with vaccination history. In addition, these studies include a distinct vaccination protocol of pregnant mares on stud farms. However, in a study of Bresgen et al. in 2012,[192] both groups of mares vaccinated twice with a MLV (5. and 8 month of pregnancy) or three times with a IWVV (5., 7. and 9. month of pregnancy) showed moderately increasing antibody concentrations with highest concentrations at the time of foaling.[192] This is in contrast with our low seroconversion rate short lived immune response.

Natural exposure to EHV-1 in the control group resulted in a strong but short-lived antibody response in one horse 4 months after the EHV-1 abortion occurred. These results are in agreement with prior investigations, that also showed that humoral immunity was only present temporarily in most horses after EHV-1 infection.[189,190] Whereas virus -neutralizing (VN) antibodies are reported to achieve a longer protection up to one year, complement-fixing (CF) antibodies only last for three months and show cross reactivity with EHV-4.[152] Both antibody types are reported to be produced within two weeks after infection[152] but EHV-1 reinfection with accompanying disease may occur after 3-6 months.[102,103] The ELISA used in our study detects both VN antibodies as well as CF antibodies.

Interestingly a second horse in the control group showed a strong rise in EHV-1 antibody titers which was unrelated to a known exposure. However, this horse did travel to three locations at three different places in Switzerland for competition the month before the antibody rise. The horse never showed any clinical signs of disease. This is consistent with frequent previous observations, reporting seroconversion without clinical signs of upper respiratory tract infection in adult horses.[9,96]

One of the vaccinated horses showed an adverse reaction after administration of the first vaccine. The observed side effects are in in agreement with the manufacturers reports.[310] Swelling at injection side up to 5cm are very common (> 1 of 10 treated horses), frequently (> 1 but < 10 of 100 treated horses) accompanied by elevated rectal temperature up to 1.7°C for two days after vaccination. Normally, clinical signs resolve without medical treatment. However, the horse in our study showed additionally reduced general condition, inappetence and stiff gait and was treated for two days with flunixin (Mefosyl®, Zoetis Schweiz GmbH, Delémont, Switzerland,1.1 mg/kg SID, IV). These clinical signs, are uncommon (> 1 but < 10 of 10'000 treated horses) and medical treatment is recommended by the manufacturer.[310]

In conclusion we showed that an antibody response to EHV-1 vaccination only occured in approximately 8/15 animals, while EHV-4 antibodies were prevalent in the equine population irrespective of vaccination. These findings suggest, that generally, the influence of vaccination on either EHV-1 and EHV-4 antibody response is moderate. It is likely, that there are horses showing a high and possibly as well a prolonged humoral response, defined as responders, whilst the non-responders do not show a detectable humoral immune response despite regular vaccination. In every Group, irrespective of vaccination, responders and non-responders were detected, suggesting that either vaccination or natural infection is correlated with responder status but immune system and genetics of the individual animal play an important role. Interestingly, EHV-1 serological analysis showed much more non-responders compared to the high number of EHV-4 responders despite close relationship of these two alpha herpesviruses. Further immunological analysis of the horses including determination of certain pathways/molecular markers in correlation with EHV-1 specific proteins are necessary to detect difference in EHV-1 and EHV-4 responder status.

This part of the study shows the complexity of the immune system and the challenge of influencing immunologic pathways by vaccination. Although the small study population does not allow representative conclusions, it is likely that immune response against EHV-1 and EHV-4 vaccination is still not satisfying. Far more in vitro studies are necessary to understand processes of the immune systems to design effective vaccines.

4.1.3 Contamination due to DNA presence on outside of vaccine bottles

An interesting aspect of this study was the detection of EHV DNA on the vaccine bottles and hands of the researcher handling these bottles. When results became available it appeared as if almost all horses (93%) were shedding virus in their nasal secretions on the day of vaccine administration right before the vaccine was administered. As there was no plausible explanation for this phenomenon, the authors concluded that contamination of the samples must have somehow occurred. The PCR protocols and runs were reviewed, however there was no conclusive pattern that could have resulted in contamination of all pre-vaccination samples in the laboratory. When reviewing the protocols of the testing days it became evident that the vaccine bottles had been handled before the nasal swabs were taken, despite the vaccine only being administered after the swab was taken. We then tested the hypothesis of

DNA presence on the vaccine bottles that could be transferred to the hands and therefore the nasal swabs.

The EHV-vaccine bottle was positive for EHV DNA, the tetanus vaccine bottle used as negative control was negative. The measured CT-Values were lower for EHV-4 than for EHV-1, implying stronger positivity for EHV-4 compared to EHV-1 in this trial. A possible reason might be the larger amount of EHV-4 DNA in the vaccine in comparison to EHV-1 (Tab. 20). As the qPCR technique is extremely sensitive, handling the vaccine followed by taking nasal swabs likely contaminated these samples. Contamination of the hands after drawing vaccine in the syringe showed a positive result for EHV-4 but not EHV-1. However, accumulation of EHV-1/4 DNA likely occurred by repeating the procedure with multiple vials consecutively, which happened during the field trial, but was not tested in this experiment. Similar observations have already been made in other studies. Positive nasal swabs on the day of vaccination with an Infectious Bovine Rhinotracheitis (IBR) marker vaccine have been reported in Belgium (personal communication C. Bachofen, information obtained from a meeting of European IBR reference laboratories in Paris, 2020). The contamination was verified as the strain detected in the animals was confirmed to be the vaccine strain. As the EHV-1,4 vaccine is not a marker vaccine, differentiation of natural and vaccine strains is not possible. This problem of potential contamination should be considered when planning future experiments and when interpreting results from other studies.

4.1.4 EHV-1 and EHV-4 viral shedding after vaccination

More horses (73%) were shedding EHV-1 and EHV-4 after vaccination compared to the control horses before the outbreak (20%). However, qPCR is an extremely sensitive technique, detecting smallest amounts of virus DNA.[178,303] The detected viral DNA does not necessarily confirm the presence of infective virus particles, therefore no conclusion regarding replication capacity or infectivity can be made. Several explanations for the virus shedding in the vaccination groups are possible: Transportation of the inactivated virions by antigen-presenting cells to the nasal mucosa, transmission by cell-to-cell or neuronal pathways or reactivation of latent virus infections following vaccination. Differentiation between shedding of vaccination-associated inactivated virion particles and reactivation of latent EHV infection is performed by cell culture. However, this is not possible in our study retrospectively due to the obtained sampling material which cannot be used for cell culture. Instead, comparison of the excreted virus sequence with the vaccination strain could be attempted, but was not performed.

In contrast to our study, nasal virus shedding following EHV vaccination has been described rarely in the past. Studies in bovines investigating excretion of infectious vaccine virions by PCR and cell culture after intramuscular (i.m.) vaccination with a glycoprotein E (gE) deleted bovine herpesvirus 1 (BoHV-1) strain showed that nasal shedding and subsequent transmission to non-vaccinated control animals occurred occasionally.[242] Neither in the MLV nor in the IWVV group, nasal shedding was observed in calves vaccinated at 3 months of age.

However, in 2-week-old vaccinated calves they detected nasal virus shedding in very low titers, but unvaccinated contact calves did not seroconvert. At very low titers in some calves that were vaccinated at 2 weeks of age, nasal viral shedding was detected.[242] Similar results are reported by El-Kholy et al. reporting no nasal virus shedding after i.m. vaccination with a live and killed adjuvanted gE negative BoHV-1 mutant in all vaccinated calves.[311]

A reason for the infrequent detection of viral shedding following vaccination in other studies compared to our investigation might be related to the absence of the gE gene in the vaccine strain of our study. Several studies have demonstrated the importance of gE for cell-to-cell spread in vitro.[312] In cell culture, gE-negative alpha herpesvirus strains can only spread by passing the extracellular fluid and entering a neighboring cell, which is a less efficient way of spreading compared to cell-to-cell spread.[312-315] EHV-1 and EHV-4 uses similar glycoproteins as other alpha herpesviruses (e.g. herpes simplex virus, HSV; pseudorabies virus, PRV and BoHV) to bind to permissive cells and cell-to cell spread.[67] The IWVV vaccine used in our study contained EHV-1 and EHV-4 strains without viral structural gE deletion and therefore cell-to-cell spread may have occurred more efficiently resulting in more frequent nasal virus shedding.

Another impact of the viral structural gE is the influence on neuronal spreading. In vivo studies showed limited neuronal spread in several hosts for gE-negative strains of PRV and HSV.[316-318] For BoHV-1, which is hardly microinvasive, difference between spread of wild-type virus and gE-negative mutant were detected in terms of the amount and duration of virus infection in several tissues.[319] However, for EHV-1 and EHV-4 predominantly intra-axonal transmission is reported to be the most efficient transmission pathway of all transmission routes.[58] Therefore, neuronal spreading supported by gE might be a reason for the relatively frequent virus shedding compared to previous studies.

Our investigations showed that various horses with EHV-1 antibodies at pre-study status had positive PCR results in the nasal swabs after vaccination. One of these horses displayed a strong positive EHV-1 CT value, too high to suggest relocation of the vaccine strain as a cause for the viral shedding. Hence reactivation of latent EHV-1 infection by vaccination is suggested in this case. Although the reactivation mechanism of latent virus infection by vaccination is unclear, this theory could explain the suggested observation of an increased likelihood of EHM development with more frequent vaccination.[18,146] None of the horses in our study developed clinical signs of upper respiratory tract disease or EHM and therefore no conclusion regarding neurological disease and vaccination can be drawn from this study.

Reasons for more frequent EHV-1/4 virus shedding after winter vaccination include seasonal aspects such as temperature, time spent indoors, as well as cumulative effects of increased virus shedding after administration of repeated vaccinations.

There are several limitations of this study, including a small sample size, and the lack of a control group as natural EHV-1 infection in the control group occurred. Additionally, results of viral shedding before vaccination are lacking due to contamination of the samples with DNA from the administered vaccine.Overall, there are no studies available on equine herpesvirus detection following vaccination to compare our results to. The assumption of repeated vaccination leading to increased virus reactivation and shedding following vaccination needs further investigation. In conclusion, vaccination may lead to transient virus shedding following vaccination.

4.2 Part B – Screening for equine herpesviruses in healthy horses

Screening for equine herpes viral shedding of 68 sound, adult horses from Switzerland (65) and Germany (3) showed frequent shedding of EHV-2. In contrast, other gamma herpesviruses, such as EHV-5 and AHV-5 are excreted rarely. Furthermore, none of the horses was positive for equine alpha herpesvirus such as EHV-1 or EHV-4.

The high prevalence of EHV-2 is in agreement with other worldwide investigations.[257,258] Reported prevalence of EHV-5 ranges from 0-100%, depending on geographic location, age and the investigated specimens.[26,262,263] A recent broad prevalence study of herpesviruses from the respiratory tract of Polish horses recorded 80% equine herpesvirus positive results in nasal swabs of more than 500 sound horses.[258]

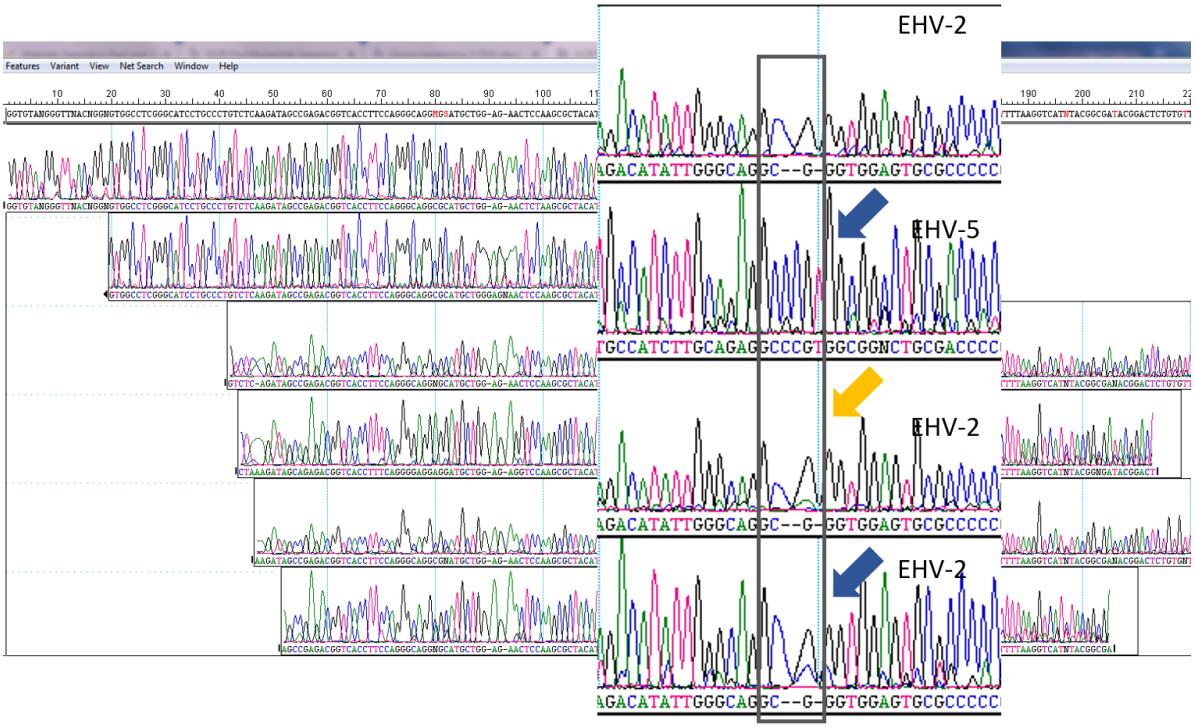
EHV-5 and EHV-2 were found more often in horses with lower airway inflammation compared to healthy horses in prior studies.[26,142,264] Investigations, considering correlation of equine gamma herpesviruses and clinical disease suggest, that EHV-2 likely induces or predisposes equids to respiratory disease.[26] Horses shedding EHV-2 were three times more likely to display clinical respiratory disease compared to non shedders.[26] The asinine herpesviruses AHV-4, AHV-5 and AHV-6 have been detected in donkeys with interstitial pneumonia, with lesions similar to EMPF.[243,299] AHV-5 in horses was reported with respiratory disorders or "poor performance syndrome"[246] as well as in two EMPF case reports with concurrent EHV-5 infection.[283] To the authors knowledge, there are no previous studies investigating range and frequency of equine gamma herpesviruses shedding in Switzerland.

In our study population, only gamma herpesviruses were detected, no alpha or beta herpes viruses were detected. This is not due to the method used, as panherpes Nested PCR is a sensitive technique to identify a broad range of herpesviruses, including alfa-, beta- and gamma herpesviruses. This method targets for a highly conserved region of the herpesviral DNA polymerase gene. Using degenerate consensus primers, PCR is able to not only detect known herpes viruses, but also novel herpesviruses without information on DNA sequence.[305] However, sequencing of PCR products is impaired by small amount of present virus. Therefore, incomplete reads and incorrect incorporation of bases by Taq DNA polymerase may occur.[305] Co-infections may often be overlooked as only the sequence of the dominant virus will be identified in a panherpesvirus PCR. Superimposed sequences and double peaks in electropherograms are indications for co-infections.[320] In our study, coinfections of gamma herpesviruses with small amounts of alpha herpesviruses can therefore not be ruled out.

In our study, coinfections, predominately EHV-2 and EHV-5, were shown in approximately 35% (14/40) of positive sequences. In this investigation, every positive result was assessed visually in an electropherogram viewer regarding likelihood of coinfection. Sequences supposed to include coinfection by electropherogram assessment were examined additionally by an alignment for illustration in the SeqMan Pro program (Fig. 30). Therefore, this study

only gives an estimation on equine herpesvirus coinfections. More precise investigations including cloning of the PCR sequences to receive two discrete sequences would be necessary.

Figure 30: Exemplary demonstration of EHV-2 with EHV-5 coinfection in samples examined by panherpes nested PCR of four sequences



Insert: alignment of the sequences with suspected coinfection due to double peaks in the electropherogram. On top, EHV-2 sequence without coinfection, followed by coinfection of EHV-5 with EHV-2 (blue arrows) and EHV-2 with either EHV-5 or AHV-5 infection (orange arrow) are presented.

Our results are in agreement with previous prevalence investigations in other countries. For example in turkey, coinfection with EHV-2 and EHV-5 occurred in 45% of examined samples.[257] In New Zealand coinfection with multiple herpesviruses (EHV-1, -2, -4, -5) in more than 50% of the virus-positive tested horses was shown.[142] Detection rates of co-infection vary depending on sampling site. A considerably lower coinfection rates of 5% in bronchoalveolar fluid (BALF) samples of horses with respiratory disease were reported in a recent study in Ethiopia.[26] EHV-5 is the suggested primary causative agent of Equine Multinodular Pulmonary Fibrosis (EMPF). However all gamma herpesviruses have the potential for inducing fibrosis and coinfection with EHV-2 and AHV-5 has been reported in approximately 20%[321] and 3% of published case reports respetively.[283,321]

Although the sampled population is not fully representative for the entire Swiss horse population, a first insight into the prevalence of equine gamma herpesviruses in Switzerland was obtained. It appears as if horses in Switzerland shed a similar range of equine gamma herpesviruses compared to reports from other countries. Also the prevalence of shedding appears similar to other countries. Further investigations including a larger sample size as well as longitudinal sampling of individual horses are necessary to obtain more information on the importance of equine gamma herpesviruses in Swiss horses.

A. References

1. Davison AJ. Herpesvirus systematics. Vet Microbiol. 2010;143(1):52-69.

2. Pellett PE, Roizman B. The family Herpesviridae: a brief introduction. In: Knipe DM, Howley PM, Griffin DE, et al. Fields Virology. 5th ed. ed. Philadelphia: Lippincott Williams & Wilkins; 2007.

3. Engels M, Ackermann M. Pathogenesis of ruminant herpesvirus infections. Veterinary Microbiology. 1996;53(1-2):3-15.

4. Davison AJ, Arvin A, Campadelli-Fiume G, et al. "Overview of classification" in *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis, eds.*2007.

5. Ostlund EN. The equine herpesviruses. Vet Clin North Am Equine Pract. 1993;9(2):283-294.

6. Crabb BSaS, M. J. Equine herpesviruses 4 (equine rhinopneumonitis virus) and 1 (equine abortion virus). Adv Virus Res. 1995;45:153-190.

7. Taniguchi A, Fukushi H, Matsumura T, Yanai T, Masegi T, Hirai K. Pathogenicity of a new neurotropic equine herpesvirus 9 (gazelle herpesvirus 1) in horses. J Vet Med Sci. 2000;62(2):215-218.

8. Davison AJ, Eberle R, Ehlers B, et al. The order Herpesvirales. Arch Virol. 2009;154(1):171-177.

9. Allen GP, Kydd JH, Slater JD, Smith KC. Equid herpesvirus 1 and equid herpesvirus 4 infections. In: Coetzer JAW, Tustin RC. Infectious Diseases of Livestock. 2nd Edition ed. Cape Town: Oxford Press; 2004.

10. Davison A, Eberle R, Desrosiers RC, Fleckenstein B, McGeoch DJ, Pellet PEea. "Herpesviridae". In: Van Regenmortel MHV, et al. Proceeding of the Seventh Report of the International Committee on Taxonomy of Viruses. Orlando: FL: AcademicPress; 2000.

11. Roizman B, Desrosiers RC, Fleckenstein B, Lopez C, Minson AC, Studdert MJ. "Herpesviridae". In: Murray FA, Faucet CM, D.H.L. B. Virus Taxonomy, Report of the International Committee on Taxonomy of Viruses. New York: NY:Springer-Verlag; 1995.

12. Agius CT, Studdert MJ. Equine herpesviruses 2 and 5: comparisons with other members of the subfamily gammaherpesvirinae. Adv Virus Res. 1994;44:357-379.

13. Pusterla N, Soboll Hussey G. Equine Herpesvirus 1 Myeloencephalopathy. Veterinary Clinics of North America Equine Practice. 2014;30:489–506.

14. Lunn DP, Davis-Poynter N, Flaminio MJ, et al. Equine herpesvirus-1 consensus statement. J Vet Intern Med. 2009;23(3):450-461.

15. Telford EA, Watson MS, McBride K, Davison AJ. The DNA sequence of equine herpesvirus-1. Virology. 1992;189(1):304-316.

16. Hartley CA, Drummer HE, Studdert MJ. The nucleotide sequence of the glycoprotein G homologue of equine herpesvirus 3 (EHV3) indicates EHV3 is a distinct equid alphaherpesvirus. Arch Virol. 1999;144(10):2023-2033.

17. Telford EA, Watson MS, Perry J, Cullinane AA, Davison AJ. The DNA sequence of equine herpesvirus-4. J Gen Virol. 1998;79 (Pt 5):1197-1203.

18. Reed SM, Toribio RE. Equine herpesvirus 1 and 4. Vet Clin North Am Equine Pract. 2004;20(3):631-642.

19. Poth T, Niedermaier G, Herrmanns W. Equine Multinodular Pulmonary Fibrosis in association with an EHV-5 infection in 5 horses. Wien Tierärztlliche Monatsschrift. 2009;96.

20. Williams KJ, Maes R, Del Piero F, et al. Equine multinodular pulmonary fibrosis: a newly recognized herpesvirus-associated fibrotic lung disease. Vet Pathol. 2007;44(6):849-862.

21. Bueno I, Pearce P, Dunowska M. Frequency of latent equine herpesvirus type-1 infection among a sample of horses in the central North Island of New Zealand. N Z Vet J. 2020;68(1):23-30.

22. Preziuso S, Sgorbini M, Marmorini P, Cuteri V. Equid alphaherpesvirus 1 from Italian Horses: Evaluation of the Variability of the ORF30, ORF33, ORF34 and ORF68 Genes. Viruses. 2019;11(9).

23. Sutton G, Garvey M, Cullinane A, et al. Molecular Surveillance of EHV-1 Strains Circulating in France during and after the Major 2009 Outbreak in Normandy Involving Respiratory Infection, Neurological Disorder, and Abortion. Viruses. 2019;11(10).

24. Castro ER, Arbiza J. Detection and genotyping of equid herpesvirus 1 in Uruguay. Rev Sci Tech. 2017;36(3):799-806.

25. Izume S, Kirisawa R, Ohya K, et al. The full genome sequences of 8 equine herpesvirus type 4 isolates from horses in Japan. J Vet Med Sci. 2017;79(1):206-212.

26. Negussie H, Gizaw D, Tesfaw L, et al. Detection of Equine Herpesvirus (EHV) -1, -2, -4 and -5 in Ethiopian Equids with and without Respiratory Problems and Genetic Characterization of EHV-2 and EHV-5 Strains. Transbound Emerg Dis. 2017;64(6):1970-1978.

27. Cruz F, Fores P, Mughini-Gras L, Ireland J, Moreno MA, Newton JR. Seroprevalence and factors associated with equine herpesvirus type 1 and 4 in Spanish Purebred horses in Spain. Vet Rec. 2016;178(16):398.

28. Pusterla N, Mapes S, Akana N, et al. Prevalence factors associated with equine herpesvirus type 1 infection in equids with upper respiratory tract infection and/or acute onset of neurological signs from 2008 to 2014. Vet Rec. 2016;178(3):70.

29. Choi E-J, Lee H-K, Lee K-H, et al. The first virus isolation and partial characterization of equine herpesvirus-4 in a horse, South Korea. Korean Journal of Veterinary Service. 2015;2(38):141-144.

30. Dunowska M, Gopakumar G, Perrott MR, et al. Virological and serological investigation of Equid herpesvirus 1 infection in New Zealand. Vet Microbiol. 2015;176(3-4):219-228.

31. Schulman ML, Becker A, van der Merwe BD, Guthrie AJ, Stout TA. Epidemiology and reproductive outcomes of EHV-1 abortion epizootics in unvaccinated Thoroughbred mares in South Africa. Equine Vet J. 2015;47(2):155-159.

32. Yildirim Y, Yilmaz V, Kirmizigul AH. Equine herpes virus type 1 (EHV-1) and 4 (EHV-4) infections in horses and donkeys in northeastern Turkey. Iran J Vet Res. 2015;16(4):341-344.

33. Ploszay G, Rola J, Larska M, Zmudzinski JF. First report on equine herpesvirus type 4 isolation in Poland--evaluation of diagnostic tools. Pol J Vet Sci. 2013;16(3):493-500.

34. Pusterla N, Mapes S, David Wilson W. Prevalence of latent alpha-herpesviruses in Thoroughbred racing horses. Vet J. 2012;193(2):579-582.

35. Fuentealba NA, Sguazza GH, Eöry ML, Valera AR, Pecoraro MR, Galosi CM. Genomic study of Argentinean Equid herpesvirus 1 strains. Rev Argent Microbiol. 2011;43(4):273-277.

36. Tsujimura K, Oyama T, Katayama Y, et al. Prevalence of equine herpesvirus type 1 strains of neuropathogenic genotype in a major breeding area of Japan. J Vet Med Sci. 2011;73(12):1663-1667.

37. Maeda K, Kai K, Matsumura T. Genomic diversity among equine herpesvirus-4 field isolates. J Vet Med Sci. 2005;67(6):555-561.

38. Oladunni FS, Horohov DW, Chambers TM. EHV-1: A Constant Threat to the Horse Industry. Front Microbiol. 2019;10:2668.

39. Negussie H, Gizaw D, Tessema TS, Nauwynck HJ. Equine Herpesvirus-1 Myeloencephalopathy, an Emerging Threat of Working Equids in Ethiopia. Transbound Emerg Dis. 2017;64(2):389-397.

40. Dunowska M. A review of equid herpesvirus 1 for the veterinary practitioner. Part B: pathogenesis and epidemiology. N Z Vet J. 2014;62(4):179-188.

41. Traub-Dargatz JL, Pelzel-McCluskey AM, Creekmore LH, et al. Case-control study of a multistate equine herpesvirus myeloencephalopathy outbreak. J Vet Intern Med. 2013;27(2):339-346.

42. Walter J, Seeh C, Fey K, Bleul U, Osterrieder N. Clinical observations and management of a severe equine herpesvirus type 1 outbreak with abortion and encephalomyelitis. Acta Vet Scand. 2013;55:19.

43. Matczuk AK, Skarbek M, Jackulak NA, Bażanów BA. Molecular characterisation of equid alphaherpesvirus 1 strains isolated from aborted fetuses in Poland. Virol J. 2018;15(1):186.

44. Anagha G, Gulati BR, Riyesh T, Virmani N. Genetic characterization of equine herpesvirus 1 isolates from abortion outbreaks in India. Arch Virol. 2017;162(1):157-163.

45. Stasiak K, Dunowska M, Hills SF, Rola J. Genetic characterization of equid herpesvirus type 1 from cases of abortion in Poland. Arch Virol. 2017;162(8):2329-2335.

46. Stasiak K, Rola J, Ploszay G, Socha W, Zmudzinski JF. Detection of the neuropathogenic variant of equine herpesvirus 1 associated with abortions in mares in Poland. BMC Vet Res. 2015;11:102.

47. Damiani AM, de Vries M, Reimers G, Winkler S, Osterrieder N. A severe equine herpesvirus type 1 (EHV-1) abortion outbreak caused by a neuropathogenic strain at a breeding farm in northern Germany. Vet Microbiol. 2014;172(3-4):555-562.

48. Turan N, Yildirim F, Altan E, et al. Molecular and pathological investigations of EHV-1 and EHV-4 infections in horses in Turkey. Res Vet Sci. 2012;93(3):1504-1507.

49. Cano A, Galosi CM, Martin Ocampos GP, et al. Equine herpesvirus 1: characterisation of the first strain isolated in Colombia. Rev Sci Tech. 2008;27(3):893-897.

50. Molinková D, Celer V, Jahn P. Isolation and partial characterization of equine herpesvirus type 1 in Czechia. Folia Microbiol (Praha). 2004;49(5):605-611.

51. Barrandeguy ME, Lascombes F, Llorente J, Houssay H, Fernandez F. High case-rate Equine herpesvirus-1 abortion outbreak in vaccinated polo mares in Argentina. Equine Veterinary Education. 2002;14(3):132-135.

52. Henninger RW, Reed SM, Saville WJ, et al. Outbreak of neurologic disease caused by equine herpesvirus-1 at a university equestrian center. J Vet Intern Med. 2007;21(1):157-165.

53. Goehring LS, Wagner B, Bigbie R, et al. Control of EHV-1 viremia and nasal shedding by commercial vaccines. Vaccine. 2010;28(32):5203-5211.

54. Burgess BA, Tokateloff N, Manning S, et al. Nasal shedding of equine herpesvirus-1 from horses in an outbreak of equine herpes myeloencephalopathy in Western Canada. J Vet Intern Med. 2012;26(2):384-392.

55. Mori E, Lara MoC, Cunha EM, et al. Molecular characterization of Brazilian equid herpesvirus type 1 strains based on neuropathogenicity markers. Braz J Microbiol. 2015;46(2):565-570.

56. McFadden AM, Hanlon D, McKenzie RK, et al. The first reported outbreak of equine herpesvirus myeloencephalopathy in New Zealand. N Z Vet J. 2016;64(2):125-134.

57. Taouji S, Collobert C, Gicquel B, et al. Detection and isolation of equine herpesviruses 1 and 4 from horses in Normandy: an autopsy study of tissue distribution in relation to vaccination status. J Vet Med B Infect Dis Vet Public Health. 2002;49(8):394-399.

58. Rajcani J, Durmanova V. Mechanisms of replication of alpha- and betaherpesviruses and their pathogenesis. Bratisl Lek Listy. 2001;102(11):505-514.

59. Edington N, Bridges CG, Huckle A. Experimental reactivation of equid herpesvirus 1 (EHV 1) following the administration of corticosteroids. Equine Vet J. 1985;17(5):369-372.

60. Foote CE, Gilkerson JR, Whalley JM, Love DN. Seroprevalence of equine herpesvirus 1 in mares and foals on a large Hunter Valley stud farm in years pre- and postvaccination. Aust Vet J. 2003;81(5):283-288.

61. Perdue ML, Kemp MC, Randall CC, O'Callaghan DJ. Studies of the molecular anatomy of the L-M cell strain of equine herpes virus type 1: proteins of the nucleocapsid and intact virion. Virology. 1974;59(1):201-216.

62. Turtinen LW, Allen GP. Identification of the envelope surface glycoproteins of equine herpesvirus type 1. J Gen Virol. 1982;63(2):481-485.

63. Meredith DM, Stocks JM, Whittaker GR, Halliburton IW, Snowden BW, Killington RA. Identification of the gB homologues of equine herpesvirus types 1 and 4 as disulphide-linked

heterodimers and their characterization using monoclonal antibodies. J Gen Virol. 1989;70 (Pt 5):1161-1172.

64. Roizman B. Herpesviridae. In: Fields BN, Knipe DM, Howley PM. Virology. 3rd ed. Philadelphia, PA: Lippincott-Raven; 1996.

65. Roizmann B, Desrosiers RC, Fleckenstein B, Lopez C, Minson AC, Studdert MJ. The family Herpesviridae: an update. The Herpesvirus Study Group of the International Committee on Taxonomy of Viruses. Arch Virol. 1992;123(3-4):425-449.

66. Oladunni FS, Horohov DW, Chambers TM. EHV-1: A Constant Threat to the Horse Industry. Front Microbiol. 2019b;10:2668.

67. Paillot R, Case R, Ross J, Newton R, Nugent J. Equine Herpes Virus-1: Virus, Immunity and Vaccines. Open Vet. Sci. J. 2008(2):68-91.

68. Newcomb WW, Brown JC, Booy FP, Steven AC. Nucleocapsid mass and capsomer protein stoichiometry in equine herpesvirus 1: scanning transmission electron microscopic study. J Virol. 1989;63(9):3777-3783.

69. Riaz A, Murtaz-Ul-Hasan K, Akhtar N. Recent Understanding of the Classification and Life Cycle of Herpesviruses: A Review. Science Letters. 2017;5:195-207.

70. Granzow H, Klupp BG, Fuchs W, Veits J, Osterrieder N, Mettenleiter TC. Egress of alphaherpesviruses: comparative ultrastructural study. J Virol. 2001;75(8):3675-3684.

71. Ficorilli N, Studdert MJ, Crabb BS. The nucleotide sequence of asinine herpesvirus 3 glycoprotein G indicates that the donkey virus is closely related to equine herpesvirus 1. Arch Virol. 1995;140(9):1653-1662.

72. Ata EB, Zaghawa A, Ghazy AA, Elsify A, Shaapan RM. Equine Herpes Virus Type-1 Infection: Etiology, Epidemiology, Pathogenesis, Identification and Recent Diagnosis. Asian Journal of Epidemiology. 2018;1(11):34-45.

73. Nugent J, Birch-Machin I, Smith KC, et al. Analysis of equid herpesvirus 1 strain variation reveals a point mutation of the DNA polymerase strongly associated with neuropathogenic versus nonneuropathogenic disease outbreaks. J Virol. 2006;80(8):4047-4060.

74. Smith KC, Whitwell KE, Mumford JA, Hannant D, Blunden AS, Tearle JP. Virulence of the V592 isolate of equid herpesvirus-1 in ponies. J Comp Pathol. 2000;122(4):288-297.

75. Mumford JA, Hannant D, Jessett DM, O'Neil T, Smith KC, Ostlund EN. "Equine infectious diseases VII". presented at: Proceedings of the Seventh International Conference on Equine Infectious Diseases1994; Newmarket.

76. Crabb BS, Allen GP, Studdert MJ. Characterization of the major glycoproteins of equine herpesviruses 4 and 1 and asinine herpesvirus 3 using monoclonal antibodies. J Gen Virol. 1991;72 (Pt 9):2075-2082.

77. Crabb BS, Nagesha HS, Studdert MJ. Identification of equine herpesvirus 4 glycoprotein G: a type-specific, secreted glycoprotein. Virology. 1992;190(1):143-154.

78. Dimock WW, Edwards PR. Is there a filterable virus of abortion in mares. Kentucky Agric. Exp. Station Bull. 1933(333):297-301.

79. Ferrera AM. Virus abortion of mares in Spain. Trabajos Inst. de Biol. Anim. 1950;9:139-197

80. Hensel L, Donath C. First isolation of equine abortion virus from aborted fetus in Western Germany. Dtsch Tierarztl Wochenschr. 1964;71(16):421-424.

81. O'Callaghan DJ, Osterrieder. Equine Herpes Virus. In: Granoff A, Webster RG. Encyclopedia of Virology. 2nd ed. USA: Academic Press; 1999.

82. Foote CE, Love DN, Gilkerson JR, Wellington JE, Whalley JM. EHV-1 and EHV-4 infection in vaccinated mares and their foals. Vet Immunol Immunopathol. 2006;111(1-2):41-46.

83. Marenzoni ML, Passamonti F, Cappelli K, et al. Clinical, serological and molecular investigations of EHV-1 and EHV-4 in 15 unweaned thoroughbred foals. Vet Rec. 2008;162(11):337-341.

84. Donald JJ. Epidemiology and Diagnosis of Equid Herpesviruses 1 and 4 in horses in New Zealand: Massey University; 1998.

85. Gilkerson JR, Love DN, Drummer HE, Studdert MJ, Whalley JM. Seroprevalence of equine herpesvirus 1 in thoroughbred foals before and after weaning. Aust Vet J. 1998;76(10):677-682.

86. Gilkerson JR, Whalley JM, Drummer HE, Studdert MJ, Love DN. Epidemiology of EHV-1 and EHV-4 in the mare and foal populations on a Hunter Valley stud farm: are mares the source of EHV-1 for unweaned foals. Vet Microbiol. 1999;68(1-2):27-34.

87. Wood JL, Newton JR, Chanter N, Mumford JA. Association between respiratory disease and bacterial and viral infections in British racehorses. J Clin Microbiol. 2005;43(1):120-126.

88. Brown JA, Mapes S, Ball BA, Hodder AD, Liu IK, Pusterla N. Prevalence of equine herpesvirus-1 infection among Thoroughbreds residing on a farm on which the virus was endemic. J Am Vet Med Assoc. 2007;231(4):577-580.

89. Dunowska M, Wilks CR, Studdert MJ, Meers J. Viruses associated with outbreaks of equine respiratory disease in New Zealand. N Z Vet J. 2002;50(4):132-139.

90. Dunowska M, Wilks CR, Studdert MJ, Meers J. Equine respiratory viruses in foals in New Zealand. N Z Vet J. 2002;50(4):140-147.

91. Lang A, de Vries M, Feineis S, Müller E, Osterrieder N, Damiani AM. Development of a peptide ELISA for discrimination between serological responses to equine herpesvirus type 1 and 4. J Virol Methods. 2013;193(2):667-673.

92. Crabb BS, MacPherson CM, Reubel GH, Browning GF, Studdert MJ, Drummer HE. A type-specific serological test to distinguish antibodies to equine herpesviruses 4 and 1. Arch Virol. 1995;140(2):245-258.

93. Foote CE, Love DN, Gilkerson JR, Whalley JM. Detection of EHV-1 and EHV-4 DNA in unweaned Thoroughbred foals from vaccinated mares on a large stud farm. Equine Vet J. 2004;36(4):341-345.

94. Gilkerson JR, Love DN, Whalley JM. Serological evidence of equine herpesvirus 1 (EHV-1) infection in Thoroughbred foals 30-120 days of age. Australian Equine Veterinarian. 1997;15(3):128-134.

95. Aharonson-Raz K, Davidson I, Porat Y, Altory A, Klement E, Steinmann A. Seroprevalence and rate of infection of equine influenza virus (H_3N_8 and H_7N_7) and equine herpesvirus (1 and 4) in the horse population in Israel. J. Equine Vet. Sci. 2014(34):828-832.

96. Allen G. "Respiratory infections by equine herpesvirus types 1 and 4". Equine Respiratory Diseases. Ithaca, NY: International Veterinary Information Service; 2002.

97. Doll ER, Bryans JT. Incubation periods for abortion in equine viral rhinopneumonitis. J Am Vet Med Assoc. 1962;140:351-354.

98. Doll ER, Bryans JT. Epizootiology of equine viral rhinopneumonitis. J Am Vet Med Assoc. 1963;142:31-37.

99. Allen GP, Bryans JT. Molecular epizootiology, pathogenesis, and prophylaxis of equine herpesvirus-1 infections. Prog Vet Microbiol Immunol. 1986;2:78-144.

100. Hartley CA, Wilks CR, Studdert MJ, Gilkerson JR. Comparison of antibody detection assays for the diagnosis of equine herpesvirus 1 and 4 infections in horses. Am J Vet Res. 2005;66(5):921-928.

101. Patel JR, Heldens J. Equine herpesviruses 1 (EHV-1) and 4 (EHV-4)--epidemiology, disease and immunoprophylaxis: a brief review. Vet J. 2005;170(1):14-23.

102. Bryans JT. On immunity to disease caused by equine herpesvirus 1. J Am Vet Med Assoc. 1969;155(2):294-300.

103. Bryans JT. Herpesviral diseases affecting reproduction in the horse. Vet Clin North Am Large Anim Pract. 1980;2(2):303-312.

104. Hatherley LI, Hayes K, Jack I. Herpes virus in an obstetric hospital. II: Asymptomatic virus excretion in staff members. Med J Aust. 1980;2(5):273-275.

105. Matsumura T, Sugiura T, Imagawa H, Fukunaga Y, Kamada M. Epizootiological aspects of type 1 and type 4 equine herpesvirus infections among horse populations. J Vet Med Sci. 1992;54(2):207-211.

106. Gilkerson JR, Jorm LR, Love DN, Lawrence GL, Whalley JM. Epidemiological investigation of equid herpesvirus-4 (EHV-4) excretion assessed by nasal swabs taken from thoroughbred foals. Vet Microbiol. 1994;39(3-4):275-283.

107. Bryant NA, Wilkie GS, Russell CA, et al. Genetic diversity of equine herpesvirus 1 isolated from neurological, abortigenic and respiratory disease outbreaks. Transbound Emerg Dis. 2018;65(3):817-832.

108. Garvey M, Lyons R, Hector RD, Walsh C, Arkins S, Cullinane A. Molecular Characterisation of Equine Herpesvirus 1 Isolates from Cases of Abortion, Respiratory and Neurological Disease in Ireland between 1990 and 2017. Pathogens. 2019;8(1).

109. Allen GP, Bolin DC, Bryant U, et al. Prevalence of latent, neuropathogenic equine herpesvirus-1 in the Thoroughbred broodmare population of central Kentucky. Equine Vet J. 2008;40(2):105-110.

110. Gilkerson J, Jorm LR, Love DN, Lawrence GL, Whalley JM. Epidemiological investigation of equid herpesvirus-4 (EHV-4) excretion assessed by nasal swabs taken from thoroughbred foals. Vet Microbiol. 1994;39(3-4):275-283.

111. Vissani MA, Becerra ML, Olguín Perglione C, Tordoya MS, Miño S, Barrandeguy M. Neuropathogenic and non-neuropathogenic genotypes of Equid Herpesvirus type 1 in Argentina. Vet Microbiol. 2009;139(3-4):361-364.

112. Pronost S, Léon A, Legrand L, et al. Neuropathogenic and non-neuropathogenic variants of equine herpesvirus 1 in France. Vet Microbiol. 2010;145(3-4):329-333.

113. Fritsche AK, Borchers K. Detection of neuropathogenic strains of Equid Herpesvirus 1 (EHV-1) associated with abortions in Germany. Vet Microbiol. 2011;147(1-2):176-180.

114. Perkins GA, Goodman LB, Tsujimura K, et al. Investigation of the prevalence of neurologic equine herpes virus type 1 (EHV-1) in a 23-year retrospective analysis (1984-2007). Vet Microbiol. 2009;139(3-4):375-378.

115. Smith KL, Allen GP, Branscum AJ, et al. The increased prevalence of neuropathogenic strains of EHV-1 in equine abortions. Vet Microbiol. 2010;141(1-2):5-11.

116. Welch HM, Bridges CG, Lyon AM, Griffiths L, Edington N. Latent equid herpesviruses 1 and 4: detection and distinction using the polymerase chain reaction and co-cultivation from lymphoid tissues. J Gen Virol. 1992;73 (Pt 2):261-268.

117. Edington N, Welch HM, Griffiths L. The prevalence of latent Equid herpesviruses in the tissues of 40 abattoir horses. Equine Vet J. 1994;26(2):140-142.

118. Chesters PM, Allsop R, Purewal A, Edington N. Detection of latency-associated transcripts of equid herpesvirus 1 in equine leukocytes but not in trigeminal ganglia. J Virol. 1997;71(5):3437-3443.

119. Slater JD, Borchers K, Thackray AM, Field HJ. The trigeminal ganglion is a location for equine herpesvirus 1 latency and reactivation in the horse. J Gen Virol. 1994;75 (Pt 8):2007-2016.

120. Baxi MK, Efstathiou S, Lawrence G, Whalley JM, Slater JD, Field HJ. The detection of latency-associated transcripts of equine herpesvirus 1 in ganglionic neurons. J Gen Virol. 1995;76 (Pt 12):3113-3118.

121. Smith DJ, Iqbal J, Purewal A, Hamblin AS, Edington N. In vitro reactivation of latent equid herpesvirus-1 from CD5+/CD8+ leukocytes indirectly by IL-2 or chorionic gonadotrophin. J Gen Virol. 1998;79 (Pt 12):2997-3004.

122. McCartan CG, Russell MM, Wood JL, Mumford JA. Clinical, serological and virological characteristics of an outbreak of paresis and neonatal foal disease due to equine herpesvirus-1 on a stud farm. Vet Rec. 1995;136(1):7-12.

123. Crowhurst FA, Dickinson G, Burrows R. An outbreak of paresis in mares and geldings associated with equid herpesvirus 1. Vet Rec. 1981;109(24):527-528.

124. Mumford JA. The epidemiology of Equid herpesvirus abortion: A tantalising mystery. Equine Vet. J. 1991;23:77-78.

125. Carrigan M, Cosgrove P, Kirkland P, Sabine M. An outbreak of Equid herpesvirus abortion in New South Wales. Equine Vet J. 1991;23(2):108-110.

126. Mumford JA, Rossdale PD, Jessett DM, Gann SJ, Ousey J, Cook RF. Serological and virological investigations of an equid herpesvirus 1 (EHV-1) abortion storm on a stud farm in 1985. J Reprod Fertil Suppl. 1987;35:509-518.

127. Slater J. Equine Herpesviruses. In: Sellon DC, Long MT. Equine Infectious Diseases. St. Louis, MO: WB Saunders Elsevier; 2007.

128. Noronha LE, Antczak DF. Modulation of T-cell reactivity during equine pregnancy is antigen independent. Am J Reprod Immunol. 2012;68(2):107-115.

129. Allen GP. Antemortem detection of latent infection with neuropathogenic strains of equine herpesvirus-1 in horses. Am J Vet Res. 2006;67(8):1401-1405.

130. Purewal AS, Smallwood AV, Kaushal A, Adegboye D, Edington N. Identification and control of the cis-acting elements of the immediate early gene of equid herpesvirus type 1. J Gen Virol. 1992;73 (Pt 3):513-519.

131. Tewari D, Gibson JS, Slater JD, et al. Modulation of the serological response of specific pathogen-free (EHV-free) foals to EHV-1 by previous infection with EHV-4 or a TK-deletion mutant of EHV-1. Arch Virol. 1993;132(1-2):101-120.

132. Gardiner DW, Lunn DP, Goehring LS, et al. Strain impact on equine herpesvirus type 1 (EHV-1) abortion models: viral loads in fetal and placental tissues and foals. Vaccine. 2012;30(46):6564-6572.

133. Foote CE, Love DN, Gilkerson JR, Whalley JM. Serological responses of mares and weanlings following vaccination with an inactivated whole virus equine herpesvirus 1 and equine herpesvirus 4 vaccine. Vet Microbiol. 2002;88(1):13-25.

134. Hebia-Fellah I, Léauté A, Fiéni F, et al. Evaluation of the presence of equine viral herpesvirus 1 (EHV-1) and equine viral herpesvirus 4 (EHV-4) DNA in stallion semen using polymerase chain reaction (PCR). Theriogenology. 2009;71(9):1381-1389.

135. Walter J, Balzer HJ, Seeh C, Fey K, Bleul U, Osterrieder N. Venereal shedding of equid herpesvirus-1 (EHV-1) in naturally infected stallions. J Vet Intern Med. 2012;26(6):1500-1504.

136. Kohn CW, Reed SM, Sofaly CD, et al. Transmission of EHV-1 by horses with EHV-1 myeloencephalopathy: Implications for biosecurity and review 2006.

137. Gryspeerdt AC, Vandekerckhove AP, Garré B, Barbé F, Van de Walle GR, Nauwynck HJ. Differences in replication kinetics and cell tropism between neurovirulent and non-neurovirulent EHV1 strains during the acute phase of infection in horses. Vet Microbiol. 2010;142(3-4):242-253.

138. Azab W, Lehmann MJ, Osterrieder N. Glycoprotein H and α4β1 integrins determine the entry pathway of alphaherpesviruses. J Virol. 2013;87(10):5937-5948.

139. Azab W, Osterrieder N. Glycoproteins D of equine herpesvirus type 1 (EHV-1) and EHV-4 determine cellular tropism independently of integrins. J Virol. 2012;86(4):2031-2044.

140. Kurtz BM, Singletary LB, Kelly SD, Frampton AR. Equus caballus major histocompatibility complex class I is an entry receptor for equine herpesvirus type 1. J Virol. 2010;84(18):9027-9034.

141. Pusterla N, Wilson DW, Madigan JE, Ferraro GL. Equine herpesvirus-1 myeloencephalopathy: A review of recent developments. The Veterinary Journal. 2009;180:279–289.

142. McBrearty KA, Murray A, Dunowska M. A survey of respiratory viruses in New Zealand horses. N Z Vet J. 2013;61(5):254-261.

143. Osterrieder N, Van de Walle GR. Pathogenic potential of equine alphaherpesviruses: the importance of the mononuclear cell compartment in disease outcome. Vet Microbiol. 2010;143(1):21-28.

144. Edington N, Bridges CG, Patel JR. Endothelial cell infection and thrombosis in paralysis caused by equid herpesvirus-1: equine stroke. Arch Virol. 1986;90(1-2):111-124.

145. Patel JR, Edington N, Mumford JA. Variation in cellular tropism between isolates of equine herpesvirus-1 in foals. Arch Virol. 1982;74(1):41-51.

146. Wilson WD. Equine herpesvirus 1 myeloencephalopathy. Vet Clin North Am Equine Pract. 1997;13(1):53-72.

147. Kydd JH, Slater J, Osterrieder N, et al. Third International Havemeyer Workshop on Equine Herpesvirus type 1. Equine Vet J. 2012;44(5):513-517.

148. Goodman LB, Loregian A, Perkins GA, et al. A point mutation in a herpesvirus polymerase determines neuropathogenicity. PLoS Pathog. 2007;3(11):e160.

149. Pusterla N, Mapes S, Wademan C, White A, Estell K, Swain E. Investigation of the role of mules as silent shedders of EHV-1 during an outbreak of EHV-1 myeloencephalopathy in California. Vet Rec. 2012;170(18):465.

150. Goehring LS, van Winden SC, van Maanen C, Sloet van Oldruitenborgh-Oosterbaan MM. Equine herpesvirus type 1-associated myeloencephalopathy in The Netherlands: a four-year retrospective study (1999-2003). J Vet Intern Med. 2006;20(3):601-607.

151. Kydd JH, Smith KC, Hannant D, Livesay GJ, Mumford JA. Distribution of equid herpesvirus-1 (EHV-1) in respiratory tract associated lymphoid tissue: implications for cellular immunity. Equine Vet J. 1994;26(6):470-473.

152. Gibson JS, O'Neill T, Thackray A, Hannant D, Field HJ. Serological responses of specific pathogen-free foals to equine herpesvirus-1: primary and secondary infection, and reactivation. Vet Microbiol. 1992a;32(3-4):199-214.

153. Gibson JS, Slater JD, Awan AR, Field HJ. Pathogenesis of equine herpesvirus-1 in specific pathogen-free foals: primary and secondary infections and reactivation. Arch Virol. 1992b;123(3-4):351-366.

154. Gibson JS, Slater JD, Field HJ. The pathogenicity of Ab4p, the sequenced strain of equine herpesvirus-1, in specific pathogen-free foals. Virology. 1992c;189(1):317-319.

155. Crabb BSaS, M.J. "Equine rhinopneumonitis (equine herpesvirus 4) and equine abortion (equine herpesvirus 1)". In: Studdert MJ. Virus Infections of Equines. Amsterdam: Elsevier Science BV; 1996.

156. Lunn D, Holmes M, Gibson J, Field H, Kydd JH, Duffus W. Haematological changes and equine lymphocyte subpopulation kinteics during primary infection and attempted re-infection of specific pathogen free foals with EHV-1. Equ.Vet.J. 1991;23:35-40.

157. McCulloch J, Williamson SA, Powis SJ, Edington N. The effect of EHV-1 infection upon circulating leucocyte populations in the natural equine host. Vet Microbiol. 1993;37(1-2):147-161.

158. Mumford JA, Rossdale PD. Virus and its relationship to the "poor performance" syndrome. Equine Vet J. 1980;12(1):3-9.

159. Allen G, Kydd J, Slater J, Smith K. "Advances in understanding the pathogenesis, epidemiology and immunological control of equid herpes-1 (EHV-1) abortion". presented at: Proceedings of the Eighth International Conference on Equine Infectious Diseases1999; Newmarket: R&W Publications Limited.

160. Edington N, Smyth B, Griffiths L. The role of endothelial cell infection in the endometrium, placenta and foetus of equid herpesvirus 1 (EHV-1) abortions. J Comp Pathol. 1991;104(4):379-387.

161. Smith KC, Whitwell KE, Binns MM, Dolby CA, Hannant D, Mumford JA. Abortion of virologically negative foetuses following experimental challenge of pregnant pony mares with equid herpesvirus 1. Equine Vet J. 1992;24(4):256-259.

162. Smith KC, Whitwell KE, Mumford JA, Gower SM, Hannant D, Tearle JP. An immunohistological study of the uterus of mares following experimental infection by equid herpesvirus 1. Equine Vet J. 1993;25(1):36-40.

163. Reed SMaT, R. E. Equine herpesvirus 1 and 4. Vet Clin North Am Equine Pract. 2004;20(3):631-642.

164. Dimock w. The diagnosis of virus abortion in mares. J. Am. Vet. Med. Assoc. 1940;96:665-666.

165. Dimock WW, Brunner DW, Edwards PR. Equine virus abortion. Bull. Kentucky Agricultural Experiment Station. 1942;426:3-20.

166. Dixon RJ, Hartley WJ, Hutchins DR, et al. Perinatal foal mortality associated with a herpesvirus. Aust Vet J. 1978;54(3):103-105.

167. Hartley WJaD, R. J. An outbreak of foal perinatal mortality due to equid herpesvirus type 1: pathological observations. Equine Vet J. 1979;11(4):215-218.

168. Timoney P. Rhinopneumonitis and Viral Abortion. Veterinary Diagnostics Virology. St. Louis: MO: Mosby Year Book; 1992.

169. Whitwell KEaB, A. S. Pathological findings in horses dying during an outbreak of the paralytic form of Equid herpesvirus type 1 (EHV-1) infection. Equine Vet J. 1992;24(1):13-19.

170. Franklin T, Daft B, Silverman V, Powers E, Weicenbach S. Serological titers and clinical observations in equines suspected of being infected with EHV-1. Calif. Vet. 1985;39:22-24.

171. Del Piero FaW, P. A. Pulmonary vasculotropic EHV-1 infection in equids. Vet Pathol. 2001;38(4):474.

172. Hussey GS, Goehring LS, Lunn DP, et al. Experimental infection with equine herpesvirus type 1 (EHV-1) induces chorioretinal lesions. Vet Res. 2013;44:118.

173. Ballagi-Pordány A, Klingeborn B, Flensburg J, Belák S. Equine herpesvirus type 1: detection of viral DNA sequences in aborted fetuses with the polymerase chain reaction. Vet Microbiol. 1990;22(4):373-381.

174. Borchers K, Slater J. A nested PCR for the detection and differentiation of EHV-1 and EHV-4. J Virol Methods. 1993;45(3):331-336.

175. Kirisawa R, Endo A, Iwai H, Kawakami Y. Detection and identification of equine herpesvirus-1 and -4 by polymerase chain reaction. Vet Microbiol. 1993;36(1-2):57-67.

176. Lawrence GL, Gilkerson J, Love DN, Sabine M, Whalley JM. Rapid, single-step differentiation of equid herpesviruses 1 and 4 from clinical material using the polymerase chain reaction and virus-specific primers. J Virol Methods. 1994;47(1-2):59-72.

177. Mackie JT, MacLeod GA, Reubel GH, Studdert MJ. Diagnosis of equine herpesvirus 1 abortion using polymerase chain reaction. Aust Vet J. 1996;74(5):390-391.

178. Elia G, Decaro N, Martella V, et al. Detection of equine herpesvirus type 1 by real time PCR. J Virol Methods. 2006;133(1):70-75.

179. Smith KL, Li Y, Breheny P, et al. New real-time PCR assay using allelic discrimination for detection and differentiation of equine herpesvirus-1 strains with A2254 and G2254 polymorphisms. J Clin Microbiol. 2012;50(6):1981-1988.

180. Lechmann J, Schoster A, Ernstberger M, Fouché N, Fraefel C, Bachofen C. A novel PCR protocol for detection and differentiation of neuropathogenic and non-neuropathogenic equid alphaherpesvirus 1. J Vet Diagn Invest. 2019;31(5):696-703.

181. OIE. Equine rhinopneumonitis (Infection with equid herpesvirus-1 and -4). OIE Terrestrial Manual 2017 Chapter 2.5.9. 2017.

182. Kydd JH, Smith KC, Hannant D, Livesay GJ, Mumford JA. Distribution of equid herpesvirus-1 (EHV-1) in respiratory tract associated lymphoid tissue: implications for cellular immunity. Equine Vet J. 1994a;26(6):470-473.

183. Kydd JH, Smith KC, Hannant D, Livesay GJ, Mumford JA. Distribution of equid herpesvirus-1 (EHV-1) in the respiratory tract of ponies: implications for vaccination strategies. Equine Vet J. 1994b;26(6):466-469.

184. Johnstone S, Barsova J, Campos I, Frampton AR. Equine herpesvirus type 1 modulates inflammatory host immune response genes in equine endothelial cells. Vet Microbiol. 2016;192:52-59.

185. Sarkar S, Balasuriya UB, Horohov DW, Chambers TM. Equine herpesvirus-1 suppresses type-I interferon induction in equine endothelial cells. Vet Immunol Immunopathol. 2015;167(3-4):122-129.

186. Sarkar S, Balasuriya UB, Horohov DW, Chambers TM. The neuropathogenic T953 strain of equine herpesvirus-1 inhibits type-I IFN mediated antiviral activity in equine endothelial cells. Vet Microbiol. 2016;183:110-118.

187. Oladunni FS, Sarkar S, Reedy S, Balasuriya UBR, Horohov DW, Chambers TM. Absence of relationship between type-I interferon suppression and neuropathogenicity of EHV-1. Vet Immunol Immunopathol. 2018;197:24-30.

188. Oladunni FS, Sarkar S, Reedy S, Balasuriya UBR, Horohov DW, Chambers TM. Equid Herpesvirus 1 Targets the Sensitization and Induction Steps To Inhibit the Type I Interferon Response in Equine Endothelial Cells. J Virol. 2019a;93(23).

189. van der Meulen KM, Favoreel HW, Pensaert MB, Nauwynck HJ. Immune escape of equine herpesvirus 1 and other herpesviruses of veterinary importance. Vet Immunol Immunopathol. 2006;111(1-2):31-40.

190. Hannant D, Jessett DM, O'Neill T, Dolby CA, Cook RF, Mumford JA. Responses of ponies to equid herpesvirus-1 ISCOM vaccination and challenge with virus of the homologous strain. Res Vet Sci. 1993;54(3):299-305.

191. Dunowska M. A review of equid herpesvirus 1 for the veterinary practitioner. Part A: clinical presentation, diagnosis and treatment. N Z Vet J. 2014;62(4):171-178.

192. Bresgen C, Lämmer M, Wagner B, Osterrieder N, Damiani AM. Serological responses and clinical outcome after vaccination of mares and foals with equine herpesvirus type 1 and 4 (EHV-1 and EHV-4) vaccines. Vet Microbiol. 2012;160(1-2):9-16.

193. Allen GPaY, M. R. Use of lambda gt11 and monoclonal antibodies to map the genes for the six major glycoproteins of equine herpesvirus 1. J Virol. 1987;61(8):2454-2461.

194. Perkins G, Babasyan S, Stout AE, et al. Intranasal IgG4/7 antibody responses protect horses against equid herpesvirus-1 (EHV-1) infection including nasal virus shedding and cell-associated viremia. Virology. 2019;531:219-232.

195. Wagner B, Goodman LB, Babasyan S, et al. Antibody and cellular immune responses of naïve mares to repeated vaccination with an inactivated equine herpesvirus vaccine. Vaccine. 2015;33(42):5588-5597.

196. Breathnach CC, Yeargan MR, Sheoran AS, Allen GP. The mucosal humoral immune response of the horse to infective challenge and vaccination with equine herpesvirus-1 antigens. Equine Vet J. 2001;33(7):651-657.

197. Breathnach CC, Yeargan MR, Timoney JF, Allen GP. Detection of equine herpesvirus-specific effector and memory cytotoxic immunity in the equine upper respiratory tract. Vet Immunol Immunopathol. 2006;111(1-2):117-125.

198. O'Neill T, Kydd JH, Allen GP, Wattrang E, Mumford JA, Hannant D. Determination of equid herpesvirus 1-specific, CD8+, cytotoxic T lymphocyte precursor frequencies in ponies. Vet Immunol Immunopathol. 1999;70(1-2):43-54.

199. Zoetis, Inventor. Duvaxyn ® EHV-1,4 ad us. vet.2017.

200. AAEP. Vaccinations for adult horses. 2020.

201. Zoetis, Inventor. Pneumabort K®+1B2020.

202. Bryans JT, Allen GP. Application of a chemically inactivated, adjuvanted vaccine to control abortigenic infection of mares by equine herpesvirus I. Dev Biol Stand. 1982;52:493-498.

203. Tengelsen LA, Yamini B, Mullaney TP, et al. A 12-year retrospective study of equine abortion in Michigan. J Vet Diagn Invest. 1997(9):303-306.

204. Giles RC, Donahue JM, Hong CB, et al. Causes of abortion, stillbirth, and perinatal death in horses: 3,527 cases (1986-1991). J Am Vet Med Assoc. 1993;203(8):1170-1175.

205. Hong CB, Donahue JM, Giles RC, et al. Equine abortion and stillbirth in central Kentucky during 1988 and 1989 foaling seasons. J Vet Diagn Invest. 1993;5(4):560-566.

206. Allen GP, Yeargan MR, Turtinen LW, Bryans JT. A new field strain of equine abortion virus (equine herpesvirus-1) among Kentucky horses. Am J Vet Res. 1985;46(1):138-140.

207. MSD, Inventor. Prevaccinol®2009.

208. Patel JR, Földi J, Bateman H, Williams J, Didlick S, Stark R. Equid herpesvirus (EHV-1) live vaccine strain C147: efficacy against respiratory diseases following EHV types 1 and 4 challenges. Vet Microbiol. 2003;92(1-2):1-17.

209. Goodman LB, Wagner B, Flaminio MJ, et al. Comparison of the efficacy of inactivated combination and modified-live virus vaccines against challenge infection with neuropathogenic equine herpesvirus type 1 (EHV-1). Vaccine. 2006;24(17):3636-3645.
210. Mayr A, Pette J, Petzoldt K, Wagener K. Studies on the development of a live vaccine against rhinopneumonitis (mare abortion) of horses. Zentralbl Veterinarmed B. 1968;15(3):406-418.
211. Witherspoon DM. Vaccination against equine herpesvirus 1 and equine influenza infection. Vet Rec. 1984;115(14):363.
212. Ingelheim B, Inventor. Rhinomune®2019.
213. Mumford JA, Hannant D, Jessett DM, O'Neil T, Smith KC, Ostlund EN. Abortigenic and neurological disease caused by experimental infection with equid herpesvirus-1. In: Nakajima H, Plowright, W. Equine infectious diseases VII. Newmarket: R&W Publications; 1994.
214. Dolby CA, Hannant D, Mumford JA. Response of ponies to adjuvanted EHV-1 whole virus vaccine and challenge with virus of the homologous strain. Br Vet J. 1995;151(1):27-37.
215. Kydd JH, Townsend HG, Hannant D. The equine immune response to equine herpesvirus-1: the virus and its vaccines. Vet Immunol Immunopathol. 2006a;111(1-2):15-30.
216. Allen G, Yeargan M, Costa LR, Cross R. Major histocompatibility complex class I-restricted cytotoxic T-lymphocyte responses in horses infected with equine herpesvirus 1. J Virol. 1995;69(1):606-612.
217. Kydd JH, Wattrang E, Hannant D. Pre-infection frequencies of equine herpesvirus-1 specific, cytotoxic T lymphocytes correlate with protection against abortion following experimental infection of pregnant mares. Vet Immunol Immunopathol. 2003;96(3-4):207-217.
218. Minke JM, Fischer L, Baudu P, et al. Use of DNA and recombinant canarypox viral (ALVAC) vectors for equine herpes virus vaccination. Vet Immunol Immunopathol. 2006;111(1-2):47-57.
219. Heldens JG, Hannant D, Cullinane AA, et al. Clinical and virological evaluation of the efficacy of an inactivated EHV1 and EHV4 whole virus vaccine (Duvaxyn EHV1,4). Vaccination/challenge experiments in foals and pregnant mares. Vaccine. 2001;19(30):4307-4317.
220. Soboll G, Breathnach CC, Kydd JH, Hussey SB, Mealey RM, Lunn DP. Vaccination of ponies with the IE gene of EHV-1 in a recombinant modified live vaccinia vector protects against clinical and virological disease. Vet Immunol Immunopathol. 2010;135(1-2):108-117.
221. Soboll G, Hussey SB, Whalley JM, et al. Antibody and cellular immune responses following DNA vaccination and EHV-1 infection of ponies. Vet Immunol Immunopathol. 2006;111(1-2):81-95.
222. Burrows R, Goodridge D, Denyer MS. Trials of an inactivated equid herpesvirus 1 vaccine: challenge with a subtype 1 virus. Vet Rec. 1984;114(15):369-374.
223. Patel JR, Bateman H, Williams J, Didlick S. Derivation and characterisation of a live equid herpes virus-1 (EHV-1) vaccine to protect against abortion and respiratory disease due to EHV-1. Vet Microbiol. 2003a.;91(1):23-39.
224. Cornick J, Martens J, Martens R, Crandell R, McConnell S, Kit S. Safety and efficacy of a thymidine kinase negative equine herpesvirus-1 vaccine in young horses. Can J Vet Res. 1990;54(2):260-266.
225. Slater JD, Gibson JS, Field HJ. Pathogenicity of a thymidine kinase-deficient mutant of equine herpesvirus 1 in mice and specific pathogen-free foals. J Gen Virol. 1993;74 (Pt 5):819-828.
226. Minke JM, Flore PH, Vaarten J. An inactivated EHV-1 and EHV-4 containing vaccine reduces clinical signs in horses infected experimentally with EHV-1 or EHV-4 six months after a single vaccination. In: J.F. W, Wernery U, Mumford JA, Kaaden O-R. Equine Infectious Diseases VIII: Proceedings of the 8th International Conference on Equine Infectious Diseases. Newmarket: R&W Publications; 1998.
227. Patel JR, Földi J, Bateman H, Williams J, Didlick S, Stark R. Equid herpesvirus (EHV-1) live vaccine strain C147: efficacy against respiratory diseases following EHV types 1 and 4 challenges. Vet Microbiol. 2003b.;92(1-2):1-17.

228. Mohd-Azmi ML, Gibson JS, Rixon FJ. Protection of specific pathogen free (Spf) Foals from severe equine herpesvirus type-1 (EHV-1) infectionfollwoing immunization with non-infectious L-particles. J Microbiol. 2002;40(3):183-192.

229. Doll ER. Immunization against viral rhinopneumonitis of horses with live virus propagated in hamsters. J Am Vet Med Assoc. 1961;139:1324-1330.

230. Doll ER, Bryans JT. Immunization of young horses against viral rhinopneumonitis. Cornell Vet. 1963a.;53:24-41.

231. Doll ER, Bryans JT. A planned infection program for immunizing mares against viral rhinopneumonitis. Cornell Vet. 1963b.;53:249-262.

232. Peacock GV. Biological requirements and control of equine rhinopneumonitis vaccine (live virus). J Am Vet Med Assoc. 1969;155(2):310-312.

233. Patel JR, Didlick S, Bateman H. Efficacy of a live equine herpesvirus-1 (EHV-1) strain C147 vaccine in foals with maternally-derived antibody: protection against EHV-1 infection. Equine Vet J. 2004;36(5):447-451.

234. Kohn CW, Fenner WR. Equine herpes myeloencephalopathy. Vet Clin North Am Equine Pract. 1987;3(2):405-419.

235. Wilson JaE, D. Neurological syndrome of rhinopneumonitis. Pror. Am. Coll. Vet. Intern. Med. 1991;9:419-421.

236. Friday PA, Scarratt WK, Elvinger F, Timoney PJ, Bonda A. Ataxia and paresis with equine herpesvirus type 1 infection in a herd of riding school horses. J Vet Intern Med. 2000;14(2):197-201.

237. Foote CE, Love DN, Gilkerson JR, et al. Serum antibody responses to equine herpesvirus 1 glycoprotein D in horses, pregnant mares and young foals. Vet Immunol Immunopathol. 2005;105(1-2):47-57.

238. Lopez AM, Hines MT, Palmer GH, Knowles DP, Alperin DC, Hines SA. Analysis of anamnestic immune responses in adult horses and priming in neonates induced by a DNA vaccine expressing the vapA gene of Rhodococcus equi. Vaccine. 2003;21(25-26):3815-3825.

239. Aaep.org: online recommendations: http://www.aaep.org/custdocs/ehmehv-1faqfinal030513.pdf 2015.

240. AAEP. Vacciantions for foals. 2020.

241. Graubner C, Gerber V, Schwarzwald C, Schoster A. Mini-Vetsuisse-Konsensus zu Prävention und Management von Equinen Herpesvirus-1 (EHV1)-Ausbrüchen

242. Strube W, Auer S, Block W, et al. A gE deleted infectious bovine rhinotracheitis marker vaccine for use in improved bovine herpesvirus 1 control programs. Vet Microbiol. 1996;53(1-2):181-189.

243. Kleiboeker SB, Schommer SK, Johnson PJ, et al. Association of two newly recognized herpesviruses with interstitial pneumonia in donkeys (Equus asinus). J Vet Diagn Invest. 2002;14(4):273-280.

244. Borchers K, Thein R, Sterner-Kock A. Pathogenesis of equine herpesvirus-associated neurological disease: a revised explanation. Equine Vet J. 2006;38(3):283-287.

245. Ackermann M. Pathogenesis of gammaherpesvirus infections. Vet Microbiol. 2006;113(3-4):211-222.

246. Fortier G, van Erck E, Pronost S, Lekeux P, Thiry E. Equine gammaherpesviruses: pathogenesis, epidemiology and diagnosis. Vet J. 2010;186(2):148-156.

247. Telford EA, Watson MS, Aird HC, Perry J, Davison AJ. The DNA sequence of equine herpesvirus 2. J Mol Biol. 1995;249(3):520-528.

248. Agius CT, Nagesha HS, Studdert MJ. Equine herpesvirus 5: comparisons with EHV2 (equine cytomegalovirus), cloning, and mapping of a new equine herpesvirus with a novel genome structure. Virology. 1992;191(1):176-186.

249. Agius CT, Crabb BS, Telford EA, Davison AJ, Studdert MJ. Comparative studies of the structural proteins and glycoproteins of equine herpesviruses 2 and 5. J Gen Virol. 1994;75 (Pt 10):2707-2717.

250. Telford EA, Studdert MJ, Agius CT, Watson MS, Aird HC, Davison AJ. Equine herpesviruses 2 and 5 are gamma-herpesviruses. Virology. 1993;195(2):492-499.

251. Franchini M, Akens M, Bracher V, von Fellenberg R. Characterisation of gamma herpesviruses in the horse by PCR. Virology. 1997;238(1):8-13.

252. Bell SA, Balasuriya UB, Gardner IA, et al. Temporal detection of equine herpesvirus infections of a cohort of mares and their foals. Vet Microbiol. 2006;116(4):249-257.

253. Sharp EL, Farrell HE, Borchers K, Holmes EC, Davis-Poynter NJ. Sequence analysis of the equid herpesvirus 2 chemokine receptor homologues E1, ORF74 and E6 demonstrates high sequence divergence between field isolates. J Gen Virol. 2007;88(Pt 9):2450-2462.

254. Dunowska M, Holloway SA, Wilks CR, Meers J. Genomic variability of equine herpesvirus-5. Arch Virol. 2000;145(7):1359-1371.

255. Reubel GH, Crabb BS, Studdert MJ. Diagnosis of equine gammaherpesvirus 2 and 5 infections by polymerase chain reaction. Arch Virol. 1995;140(6):1049-1060.

256. Torfason EG, Thorsteinsdóttir L, Torsteinsdóttir S, Svansson V. Study of equid herpesviruses 2 and 5 in Iceland with a type-specific polymerase chain reaction. Res Vet Sci. 2008;85(3):605-611.

257. Akkutay AZ, Osterrieder N, Damiani A, Tischer BK, Borchers K, Alkan F. Prevalence of equine gammaherpesviruses on breeding farms in Turkey and development of a TaqMan MGB real-time PCR to detect equine herpesvirus 5 (EHV-5). Arch Virol. 2014;159(11):2989-2995.

258. Stasiak K, Dunowska M, Rola J. Prevalence and sequence analysis of equid herpesviruses from the respiratory tract of Polish horses. Virol J. 2018;15(1):106.

259. Diallo IS, Hewitson GR, de Jong A, et al. Equine herpesvirus infections in yearlings in South-East Queensland. Arch Virol. 2008;153(9):1643-1649.

260. Nordengrahn A, Merza M, Ros C, et al. Prevalence of equine herpesvirus types 2 and 5 in horse populations by using type-specific PCR assays. Vet Res. 2002;33(3):251-259.

261. Wang L, Raidal SL, Pizzirani A, Wilcox GE. Detection of respiratory herpesviruses in foals and adult horses determined by nested multiplex PCR. Vet Microbiol. 2007;121(1-2):18-28.

262. Fortier G, Pronost S, Miszczak F, et al. Identification of equid herpesvirus-5 in respiratory liquids: a retrospective study of 785 samples taken in 2006-2007. Vet J. 2009;182(2):346-348.

263. Marenzoni ML, Coppola G, Maranesi M, et al. Age-dependent prevalence of equid herpesvirus 5 infection. Veterinary Reasearch Communication. 2010;34:703-708.

264. Fortier G, van Erck E, Fortier C, et al. Herpesviruses in respiratory liquids of horses: putative implication in airway inflammation and association with cytological features. Vet Microbiol. 2009;139(1-2):34-41.

265. Browning GF, Studdert MJ. Epidemiology of equine herpesvirus 2 (equine cytomegalovirus). J Clin Microbiol. 1987;25(1):13-16.

266. Borchers K, Ebert M, Fetsch A, Hammond T, Sterner-Kock A. Prevalence of equine herpesvirus type 2 (EHV-2) DNA in ocular swabs and its cell tropism in equine conjunctiva. Vet Microbiol. 2006;118(3-4):260-266.

267. Dunowska M, Meers J, Johnson RD, Wilks CR. Influence of equine herpesvirus type 2 infection on monocyte chemoattractant protein 1 gene transcription in equine blood mononuclear cells. Res Vet Sci. 2001;71(2):111-113.

268. Schlocker N, Gerber-Bretscher R, von Fellenberg R. Equine herpesvirus 2 in pulmonary macrophages of horses. Am J Vet Res. 1995;56(6):749-754.

269. Browning GF, Ficorilli N, Studdert MJ. Asinine herpesvirus genomes: comparison with those of the equine herpesviruses. Arch Virol. 1988;101(3-4):183-190.

270. Borchers K, Wolfinger U, Goltz M, Broll H, Ludwig H. Distribution and relevance of equine herpesvirus type 2 (EHV-2) infections. Arch Virol. 1997;142(5):917-928.

271. Ruszczyk A, Cywinska A, Banbura MW. Equine herpes virus 2 infection in horse populations in Poland. Acta Virol. 2004;48(3):189-192.

272. Wilcox GE, Raidal SI. Role of viruses in respiratory disease. 2000.

273. Pálfi V, Belák S, Molnár T. Isolation of equine herpesvirus type 2 from foals, showing respiratory symptoms. Zentralbl Veterinarmed B. 1978;25(2):165-167.

274. Fu ZF, Robinson AJ, Horner GW, Dickinson LG, Grimmett JB, Marshall RB. Respiratory disease in foals and the epizootiology of equine herpesvirus type 2 infection. N Z Vet J. 1986;34(9):152-155.

275. Sledge DG, Miller DL, Styer EL, Hydrick HA, Baldwin CA. Equine herpesvirus 2-associated granulomatous dermatitis in a horse. Vet Pathol. 2006;43(4):548-552.

276. Léon A, Fortier G, Fortier C, et al. Detection of equine herpesviruses in aborted foetuses by consensus PCR. Vet Microbiol. 2008;126(1-3):20-29.

277. Galosi CM, de la Paz VC, Fernández LC, et al. Isolation of equine herpesvirus-2 from the lung of an aborted fetus. J Vet Diagn Invest. 2005;17(5):500-502.

278. Smith KC. Herpesviral abortion in domestic animals. Vet J. 1997;153(3):253-268.

279. Borchers K, Brackmann J, Kershaw O. The mouse is not permissive for equine herpesvirus 2 (EHV-2), however viral DNA persisted in lung and spleen depending on the inoculation route. Arch Virol. 2002;147(7):1437-1444.

280. Rizvi SM, Slater JD, Wolfinger U, Borchers K, Field HJ, Slade AJ. Detection and distribution of equine herpesvirus 2 DNA in the central and peripheral nervous systems of ponies. J Gen Virol. 1997;78 (Pt 5):1115-1118.

281. Schwarz B, Klang A, Bezdekova B, Sárdi S, Kutasi O, Hoven R. Equine multinodular pulmonary fibrosis (EMPF): Five case reports. Acta Vet Hung. 2013;61(3):319-332.

282. Easton-Jones CA, Cissell DD, Mohr FC, Chigerwe M, Pusterla N. Prognostic indicators and long-term survival in 14 horses with equine multinodular pulmonary fibrosis. Equine Veterinary Education. 2019;n/a(n/a):6.

283. Back H, Kendall A, Grandón R, et al. Equine multinodular pulmonary fibrosis in association with asinine herpesvirus type 5 and equine herpesvirus type 5: a case report. Acta Vet Scand. 2012;54:57.

284. Back H, Ullman K, Leijon M, et al. Genetic variation and dynamics of infections of equid herpesvirus 5 in individual horses. J Gen Virol. 2016;97(1):169-178.

285. Dunowska M, Hardcastle MR, Tonkin FB. Identification of the first New Zealand case of equine multinodular pulmonary fibrosis. N Z Vet J. 2014;62(4):226-231.

286. Hart KA, Barton MH, Williams KJ, Flaminio MJBF, Howerth EW. Multinodular pulmonary fibrosis, pancytopenia and equine herpesvirus-5 infection in a Thoroughbred gelding. Equine Veterinary Education. 2008;20(9):470-476.

287. Lehmbecker A, Biesenbach W, König P, Schneider-Bühl L, Wohlsein P. Multinodular pulmonary fibrosis in a horse from Schleswig-Holstein. Tierarztl Prax Ausg G Grosstiere Nutztiere. 2011;39(4):237-240.

288. Niedermaier G, Poth T, Gehlen H. Clinical aspects of multinodular pulmonary fibrosis in two warmblood horses. Vet Rec. 2010;166(14):426-430.

289. Schwarz B, Gruber† A, Benetka V, et al. Concurrent T cell leukaemia and equine multinodular pulmonary fibrosis in a Hanoverian Warmblood mare. Equine Veterinary Education 24. 2012;24(4):187-192.

290. Schwarz B. Successful outcome in a case of equine multinodular pulmonaryfibrosis (EMPF) treated with valacyclovir. Equine Veterinary Education. 2013;25(8):389-392.

291. Soare T, Leeming G, Morgan R, et al. Equine multinodular pulmonary fibrosis in horses in the UK. Vet Rec. 2011;169(12):313.

292. Spelta CW, Axon JE, Begg A, et al. Equine multinodular pulmonary fibrosis in three horses in Australia. Aust Vet J. 2013;91(7):274-280.

293. Tomlinson JE, Divers TJ, McDonough SP, Thompson MS. Hypertrophic osteopathy secondary to nodular pulmonary fibrosis in a horse. J Vet Intern Med. 2011;25(1):153-157.

294. Verryken KS, V., Maes S, Borchers K, Van De Walle G, Ducatelle R, Deprez P. First report of multinodular pulmonary fibrosis associated with equine herpesvirus 5 in Belgium. Vlaams Diergeneeskundig Tijdschrift. 210;79:297-301.

295. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol. 2008;214(2):199-210.

296. Marenzoni ML, Passamonti F, Lepri E, et al. Quantification of Equid herpesvirus 5 DNA in clinical and necropsy specimens collected from a horse with equine multinodular pulmonary fibrosis. J Vet Diagn Invest. 2011;23(4):802-806.

297. Williams KJ. Gammaherpesviruses and pulmonary fibrosis: evidence from humans, horses, and rodents. Vet Pathol. 2014;51(2):372-384.

298. Wong DM, Belgrave RL, Williams KJ, et al. Multinodular pulmonary fibrosis in five horses. J Am Vet Med Assoc. 2008;232(6):898-905.

299. Vengust M, Wen X, Bienzle D. Herpesvirus-associated neurological disease in a donkey. J Vet Diagn Invest. 2008;20(6):820-823.

300. Gomez De Witte FG, Frank N, Wilkes RP, Novak JM. Association of asinine herpesvirus-5 with pyogranulomatous pneumonia in a mare. J Vet Intern Med. 2012;26(4):1064-1068.

301. Nordengrahn A, Klingeborn B, Lindholm A, Merza M. The use of a neutralizing monoclonal antibody to detect infections of equine herpesvirus type 2 (EHV-2). J Vet Diagn Invest. 2001;13(5):389-393.

302. Dynon K, Varrasso A, Ficorilli N, et al. Identification of equine herpesvirus 3 (equine coital exanthema virus), equine gammaherpesviruses 2 and 5, equine adenoviruses 1 and 2, equine arteritis virus and equine rhinitis A virus by polymerase chain reaction. Aust Vet J. 2001;79(10):695-702.

303. Diallo IS, Hewitson G, Wright L, Rodwell BJ, Corney BG. Detection of equine herpesvirus type 1 using a real-time polymerase chain reaction. J Virol Methods. 2006;131(1):92-98.

304. Drummer HE, Reynolds A, Studdert MJ, MacPherson CM, Crabb BS. Application of an equine herpesvirus 1 (EHV1) type-specific ELISA to the management of an outbreak of EHV1 abortion. Vet Rec. 1995;136(23):579-581.

305. VanDevanter DR, Warrener P, Bennett L, et al. Detection and analysis of diverse herpesviral species by consensus primer PCR. J Clin Microbiol. 1996;34(7):1666-1671.

306. Ehlers B, Borchers K, Grund C, Frölich K, Ludwig H, Buhk HJ. Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. Virus Genes. 1999;18(3):211-220.

307. Lechmann J, Bachofen C, Ackermann M. Investigation into the virome of Swiss water buffaloes: Institute of Virology, Vetsuisse faculty, University of Zürich, CH-8057 Zürich; 2016.

308. Heringa J, Argos P. Evolution of viruses as recorded by their polymerase sequences. In: Morse SS. The evolutionary biology of viruses. New York: Raven Press, Ltd.; 1994.

309. Holmes MA, Townsend HG, Kohler AK, et al. Immune responses to commercial equine vaccines against equine herpesvirus-1, equine influenza virus, eastern equine encephalomyelitis, and tetanus. Vet Immunol Immunopathol. 2006;111(1-2):67-80.

310. Zoetis, Inventor. Duvaxyn ® EHV-1,4 ad us. vet.2017.

311. El-Kholy AA, Rady DI, Abdou ER, Elseafy MM, Abdelrahman KA, Soliman H. Construction, characterization and immunogenicity of a glycoprotein E negative bovine herpesvirus-1.1 Egyptian strain "Abu-Hammad". J Virol Methods. 2013;194(1-2):74-81.

312. Mars MH, de Jong MC, van Oirschot JT. A gE-negative BHV1 vaccine virus strain cannot perpetuate in cattle populations. Vaccine. 2000;18(20):2120-2124.

313. Dingwell KS, Brunetti CR, Hendricks RL, et al. Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread in vivo and across junctions of cultured cells. J Virol. 1994;68(2):834-845.

314. Zsak L, Zuckermann F, Sugg N, Ben-Porat T. Glycoprotein gI of pseudorabies virus promotes cell fusion and virus spread via direct cell-to-cell transmission. J Virol. 1992;66(4):2316-2325.

315. Rebordosa X, Piñol J, Pérez-Pons JA, et al. Glycoprotein E of bovine herpesvirus type 1 is involved in virus transmission by direct cell-to-cell spread. Virus Res. 1996;45(1):59-68.

316. Card JP, Whealy ME, Robbins AK, Enquist LW. Pseudorabies virus envelope glycoprotein gI influences both neurotropism and virulence during infection of the rat visual system. J Virol. 1992;66(5):3032-3041.

317. Mulder WA, Jacobs L, Priem J, et al. Glycoprotein gE-negative pseudorabies virus has a reduced capability to infect second- and third-order neurons of the olfactory and trigeminal routes in the porcine central nervous system. J Gen Virol. 1994;75 (Pt 11):3095-3106.

318. Dingwell KS, Doering LC, Johnson DC. Glycoproteins E and I facilitate neuron-to-neuron spread of herpes simplex virus. J Virol. 1995;69(11):7087-7098.

319. van Engelenburg FA, Kaashoek MJ, van Oirschot JT, Rijsewijk FA. A glycoprotein E deletion mutant of bovine herpesvirus 1 infects the same limited number of tissues in calves as wild-type virus, but for a shorter period. J Gen Virol. 1995;76 (Pt 9):2387-2392.

320. Pérez-Tris J, Bensch S. Diagnosing genetically diverse avian malarial infections using mixed-sequence analysis and TA-cloning. Parasitology. 2005;131(Pt 1):15-23.

321. Scheurer L, Bachofen C, Herteman N, Hilbe M, Wolfer N, Schoster A. A case series highlighting the role of different gamma-herpesviruses in Equine Multinodular Pulmonary Fibrosis. Schweiz Arch Tierheilkd. 2020;162(4):245-256.

B. Appendix

Table 18a: Overview of all available samples from every horse (H1-H24)

Sample	Timepoint of Sampling	H1	H2	H3	H4	H5	H6	H7	H8
S	preVaccS/ preVaccBI1	x	x	x	x	x	x	x	x
S	preVaccBI2							x	x
S	1mpostVaccS/ 1mpostVaccBI2	x	x	x	x	x	x	x	x
S	2mpostVaccS/ 2mpostVaccBI2	x	x	x	x	x	x	x	x
S	3mpostVaccS/ 3mpostVaccBI2	x	x	x	x	x	x	x	x
S	4mpostVaccS 4mpostVaccBI2	x	x	x	x	x	x	x	x
S	5mpostVaccS 5mpostVaccBI2	x	x	x	x	x	x	x	x
S	preVaccW							x	x
S	1mpostVaccW	x	x	x	x		x	x	x
S	2mpostVaccW	x	x	x	x		x	x	x
S	3mpostVaccW	x	x	x	x		x	x	x
S	4mpostVaccW	x	x	x	x		x	x	x
S	5mpostVaccW	x	x	x	x		x	x	x
NS	preVaccS/ preVaccBI1	x	x	x	x	x	x		
NS	1dpostVaccS/ 1dpostVaccBI1	x	x	x	x	x	x	x	x
NS	2dpostVaccS/ 2dpostVaccBI1	x	x	x	x	x	x	x	x
NS	3dpostVaccS/ 3dpostVaccBI1	x	x	x	x	x	x	x	
NS	4dpostVaccS/ 4dpostVaccBI1	x	x	x	x	x	x	x	
NS	5dpostVaccS/ 5dpostVaccBI1	x	x	x	x	x	x	x	

Sample	Timepoint of Sampling	H1	H2	H3	H4	H5	H6	H7	H8
NS	preVaccBI2							x	x
NS	1dpostVaccBI2							x	x
NS	2dpostVaccBI2							x	x
NS	3dpostVaccBI2							x	x
NS	4dpostVaccBI2							x	
NS	5dpostVaccBI2							x	x
NS	preVaccW	x	x	x	x		x	x	x
NS	1dpostVaccW	x	x	x	x		x	x	x
NS	2dpostVaccW	x	x	x	x		x	x	x
NS	3dpostVaccW	x	x	x	x		x	x	x
NS	4dpostVaccW	x	x	x	x		x	x	x
NS	5dpostVaccW							x	x
	Total S	11	11	11	11	6	11	13	13
	Total NS	11	11	11	11	6	11	17	13

Table 18b: Overview of all available samples from every horse (H9-H16)

Sample	Timepoint of Sampling	H9	H10	H11	H12	H13	H14	H15	H16
S	preVaccS/ preVaccBI1	x	x	x	x	x	x	x	x
S	preVaccBI2	x	x	x					
S	1mpostVaccS/ 1mpostVaccBI2	x	x	x	x	x	x		x
S	2mpostVaccS/ 2mpostVaccBI2	x	x	x	x	x	x		x
S	3mpostVaccS/ 3mpostVaccBI2	x	x	x	x	x	x		x
S	4mpostVaccS 4mpostVaccBI2	x	x	x	x	x	x	x	x
S	5mpostVaccS 5mpostVaccBI2	x	x	x	x	x	x	x	x

Sample	Timepoint of Sampling	H9	H10	H11	H12	H13	H14	H15	H16
S	preVaccW	x	x	x	x	x	x		x
S	1mpostVaccW	x	x	x	x	x	x		x
S	2mpostVaccW	x	x	x	x	x	x		x
S	3mpostVaccW	x	x	x	x	x	x		x
S	4mpostVaccW	x	x	x	x	x	x		x
S	5mpostVaccW	x	x	x	x	x	x		x
NS	preVaccS/ preVaccBI1		x						x
NS	1dpostVaccS/ 1dpostVaccBI1	x	x	x					x
NS	2dpostVaccS/ 2dpostVaccBI1	x	x	x				x	x
NS	3dpostVaccS/ 3dpostVaccBI1	x	x	x				x	x
NS	4dpostVaccS/ 4dpostVaccBI1	x	x	x					x
NS	5dpostVaccS/ 5dpostVaccBI1	x	x	x					x
NS	preVaccBI2	x	x	x					
NS	1dpostVaccBI2	x	x	x					
NS	2dpostVaccBI2	x	x	x					
NS	3dpostVaccBI2	x	x	x					
NS	4dpostVaccBI2	x	x	x					
NS	5dpostVaccBI2	x	x	x					
NS	preVaccW	x	x	x	x	x	x	x	x
NS	1dpostVaccW	x	x	x	x	x	x		x
NS	2dpostVaccW	x	x	x	x	x	x		x
NS	3dpostVaccW	x	x	x	x	x	x		x
NS	4dpostVaccW	x	x	x	x	x	x		x
NS	5dpostVaccW	x	x	x	x	x	x		x

Sample	Timepoint of Sampling	H9	H10	H11	H12	H13	H14	H15	H16
	Total S	13	13	13	12	12	12	3	12
	Total NS	17	18	17	6	6	6	3	12

Table 18c: Overview of all available samples from every horse (H17-H24)

Sample	Timepoint of Sampling	H17	H18	H19	H20	H21	H22	H23	H24	Total
S	preVaccS/ preVaccBI1	x	x	x	x	x	x	x	x	24/24
S	preVaccBI2					x				6/7
S	1mpostVaccS/ 1mpostVaccBI2	x	x	x	x	x	x	x	x	23/24
S	2mpostVaccS/ 2mpostVaccBI2	x	x	x	x	x	x	x	x	23/24
S	3mpostVaccS/ 3mpostVaccBI2	x		x	x	x	x	x		21/24
S	4mpostVaccS 4mpostVaccBI2	x	x	x	x	x	x	x		23/24
S	5mpostVaccS 5mpostVaccBI2	x	x	x	x	x	x	x		23/24
S	preVaccW	x	x	x		x	x	x		15/24
S	1mpostVaccW	x	x	x		x	x	x		20/24
S	2mpostVaccW	x	x	x		x	x	x		20/24
S	3mpostVaccW	x	x	x		x	x	x		20/24
S	4mpostVaccW	x	x	x		x	x	x		20/24
S	5mpostVaccW	x	x	x		x	x	x		20/24
NS	preVaccS/ preVaccBI1	x	x				x	x	x	13/24
NS	1dpostVaccS/ 1dpostVaccBI1	x		x	x		x	x	x	18/24
NS	2dpostVaccS/ 2dpostVaccBI1	x		x	x		x	x	x	19/24
NS	3dpostVaccS/ 3dpostVaccBI1	x	x	x	x		x	x	x	19/24

Sample	Timepoint of Sampling	H17	H18	H19	H20	H21	H22	H23	H24	Total
NS	4dpostVaccS/ 4dpostVaccBI1	x	x	x	x		x	x	x	18/24
NS	5dpostVaccS/ 5dpostVaccBI1	x	x	x	x		x	x	x	18/24
NS	preVaccBI2				x					6/7
NS	1dpostVaccBI2				x					6/7
NS	2dpostVaccBI2				x					6/7
NS	3dpostVaccBI2				x					6/7
NS	4dpostVaccBI2				x					5/7
NS	5dpostVaccBI2				x					6/7
NS	preVaccW	x	x	x		x	x	x		21/24
NS	1dpostVaccW	x		x		x	x	x		19/24
NS	2dpostVaccW	x	x	x		x	x	x		20/24
NS	3dpostVaccW	x	x	x		x	x	x		20/24
NS	4dpostVaccW	x	x	x		x	x	x		20/24
NS	5dpostVaccW		x	x		x	x	x		14/24
	Total S	12	11	12	6	13	12	12	3	
	Total NS	11	9	11	11	6	12	12	6	

Table 18a-c: H: horse number; S: serum sample; NS: nasal swab; x: taken sample;
Group A-C: vaccination Groups; Group D: control Group
Group A (H12, 13, 14, 22, 23); Group B (H16, 17, 18, 19); Group C (H7, 8, 9, 10, 11, 21);
Group D: (H1, H2, H3, H4, H5, H6)
preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination;
preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
mpostVaccBI1: months after the first dose of the basic immunization (Group C only);
mpostVaccBI2: months after the second dose of the basic immunization (Group C only);
BI: Basic immunization consisting of two doses given 4 weeks apart
Total: all taken samples of every sampling timepoint referred to the total of all sampled horses
Total S: all taken serum samples referred to every horse;
Total NS: all taken nasal swabs referred to every horse

Data Tables in OD-Values corresponding to Fig. 7 (Group A-C): Longitudinal EHV-1 antibody concentrations in horses vaccinated against EHV-1/4 with different protocols (n = 15)

	H7	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**
preVaccS/preVaccBI1	0.67	0.00	0.00	0.05	0.02	0.4	0.76	0.05
preVaccBI2	0.60	0.00	0.11	0.02	0.00			
1mpostVaccS/1mpostVaccBI2	0.97	0.02	0.43	0.10	0.00	0.6	0.88	0.03
2mpostVaccS/2mpostVaccBI2	0.50	0.02	0.15	0.05	0.01	0.73	1.19	0.02
3mpostVaccS/3mpostVaccBI2	0.55	0.00	0.12	0.05	0.00	0.44	0.80	0.04
4mpostVaccS/4mpostVaccBI2	0.54	0.00	0.09	0.03	0.00	0.31	0.94	0.02
5mpostVaccS/5mpostVaccBI2	0.42	0.00	0.00	0.26	0.00	0.29	0.93	0.01
preVaccW	0.12	0.00	0.08	0.02	0.00	0.28	0.94	0.02
1mpostVaccW	0.51	0.01	0.20	0.10	0.02	0.69	0.93	0.02
2mpostVaccW	0.87	0.03	0.15	0.05	0.00	0.35	1.16	0.00
3mpostVaccW	0.55	0.09	0.15	0.14	0.00	0.54	1.34	0.01
4mpostVaccW	0.56	0.00	0.10	0.07	0.04	0.22	1.04	0.07
5mpostVaccW	0.55	0.05	0.04	0.03	0.00	0.48	1.01	0.01

	H16	**H17**	**H18**	**H19**	**H21**	**H22**	**H23**
preVaccS/preVaccBI1	0.12	0.87	0.03	0.05	0.02	0.00	0.01
preVaccBI2					0.03		
1mpostVaccS/1mpostVaccBI2	0.50	0.88	0.05	0.08	0.11	0.01	0.25
2mpostVaccS/2mpostVaccBI2	0.50	1.11	0.02	0.11	0.04	0.00	0.00
3mpostVaccS/3mpostVaccBI2	0.39	0.69		0.07	0.03	0.00	0.01
4mpostVaccS/4mpostVaccBI2	0.42	0.71	0.04	0.05	0.01	0.00	0.01
5mpostVaccS/5mpostVaccBI2	0.35	0.64	0.02	0.03	0.06	0.00	0.00
preVaccW	0.09	0.37	0.01	0.09	0.06	0.00	0.01
1mpostVaccW	0.34	0.84	0.07	0.09	0.01	0.00	0.00
2mpostVaccW	0.31	0.70	0.03	0.08	0.03	0.00	0.01
3mpostVaccW	0.47	0.76	0.04	0.07	0.05	0.01	0.00
4mpostVaccW	0.38	0.76	0.05	0.10	0.03	0.00	0.02
5mpostVaccW	0.32	0.94	0.02	0.07	0.00	0.01	0.01

H: Horse number; OD: Optical density; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination;
preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
mpostVaccBI2: months after the second dose of the basic immunization (Group C only)
Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study;
Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI;
Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 8 (Group A): Longitudinal EHV-1 antibody concentrations in horses vaccinated against EHV-1 with a basic immunization >4 years ago with regular boosters every 6 months including summer and winter during the study (n = 5)

	H12	**H13**	**H14**	**H22**	**H23**
preVaccS	0.4	0.76	0.05	0.00	0.01
1mpostVaccS	0.6	0.88	0.03	0.01	0.25
2mpostVaccS	0.73	1.19	0.02	0.00	0.00
3mpostVaccS	0.44	0.80	0.04	0.00	0.01
4mpostVaccS	0.31	0.94	0.02	0.00	0.01
5mpostVaccS	0.29	0.93	0.01	0.00	0.00
preVaccW	0.28	0.94	0.02	0.00	0.01
1mpostVaccW	0.69	0.93	0.02	0.00	0.00
2mpostVaccW	0.35	1.16	0.00	0.00	0.01
3mpostVaccW	0.54	1.34	0.01	0.01	0.00
4mpostVaccW	0.22	1.04	0.07	0.00	0.02
5mpostVaccW	0.48	1.01	0.01	0.01	0.01

H: Horse number; OD: Optical density: preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD- Values corresponding to Fig. 9 (Group B): Longitudinal EHV-1 antibody concentrations in horses with no EHV-1 vaccination history which were vaccinated twice 6 months apart in summer and winter during the study (n = 4)

	H16	**H17**	**H18**	**H19**
preVaccS	0.12	0.87	0.03	0.05
1mpostVaccS	0.50	0.88	0.05	0.08
2mpostVaccS	0.50	1.11	0.02	0.11
3mpostVaccS	0.39	0.69		0.07
4mpostVaccS	0.42	0.71	0.04	0.05
5mpostVaccS	0.35	0.64	0.02	0.03
preVaccW	0.10	0.37	0.01	0.09
1mpostVaccW	0.34	0.84	0.07	0.09
2mpostVaccW	0.31	0.70	0.03	0.08
3mpostVaccW	0.47	0.76	0.04	0.07
4mpostVaccW	0.38	0.76	0.05	0.10
5mpostVaccW	0.32	0.94	0.02	0.07

Horse number; OD: Optical density: preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 10 (Group C): Longitudinal EHV-1 antibody concentrations in horses with no EHV-1 vacination history which received a basic immunization in summer (2 doses 4 weeks apart) followed by a booster vaccine 6 months later, in winter (n = 6)

	H7	**H8**	**H9**	**H10**	**H11**	**H21**
preVaccBI1	0.67	0.00	0.00	0.05	0.02	0.02
preVaccBI2	0.60	0.00	0.11	0.02	0.00	0.03
1mpostVaccBI2	0.97	0.02	0.43	0.10	0.00	0.11
2mpostVaccBI2	0.50	0.02	0.15	0.05	0.01	0.04
3mpostVaccBI2	0.55	0.00	0.12	0.05	0.00	0.03
4mpostVaccBI2	0.54	0.00	0.09	0.03	0.00	0.01
5mpostVaccBI2	0.42	0.00	0.00	0.26	0.00	0.06
preVaccW	0.12	0.00	0.08	0.02	0.00	0.06
1mpostVaccW	0.51	0.01	0.20	0.10	0.02	0.01
2mpostVaccW	0.87	0.03	0.15	0.05	0.00	0.03
3mpostVaccW	0.55	0.09	0.15	0.14	0.00	0.05
4mpostVaccW	0.56	0.00	0.10	0.07	0.04	0.03
5mpostVaccW	0.55	0.05	0.04	0.03	0.00	0.00

H: Horse number; OD: Optical density; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; mpostVaccBI2: months after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 11: Longitudinal EHV-1 antibody concentrations in control Group D (n = 5)

	H1	**H2**	**H3**	**H4**	**H6**
preVaccS	0.00	0.03	0.15	0.25	0.01
1mpostVaccS	0.00	0.15	0.37	0.39	0.00
2mpostVaccS	0.00	0.00	0.13	0.13	0.00
3mpostVaccS	0.45	0.02	0.14	0.50	0.04
4mpostVaccS	0.17	0.05	0.15	1.72	0.38
5mpostVaccS	0.32	0.05	0.16	1.57	0.36
preVaccW	Missing sample	Missing sample	Missing sample	Missing sample	Missing sample
1mpostVaccW	0.00	0.00	0.04	1.27	0.11
2mpostVaccW	0.06	0.03	1.11	0.73	0.14
3mpostVaccW	0.05	0.01	0.85	0.67	0.02
4mpostVaccW	0.03	0.03	0.79	0.50	0.11
5mpostVaccW	0.04	0.02	0.68	0.42	0.16

H: Horse number; OD: Optical density; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS; positive results: > 0.2; negative results: < 0.1; non-interpretable

OD-Values corresponding to Fig. 12 (Group A-C): Longitudinal EHV-4 antibody concentrations in horses vaccinated against EHV-1/4 with different protocols (n = 15)

	H7	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**
preVaccS/preVaccBI1	2.37	1.30	1.34	1.36	1.77	2.06	2.00	1.76
preVaccBI2	2.28	0.62	1.40	0.35	1.92			
1mpostVaccS/1mpostVaccBI2	3.03	2.92	2.07	1.90	2.62	2.11	1.61	1.81
2mpostVaccS/2mpostVaccBI2	2.22	2.00	1.53	1.32	1.73	2.85	2.90	1.64
3mpostVaccS/3mpostVaccBI2	2.24	1.80	1.62	1.07	1.67	1.86	2.12	1.74
4mpostVaccS/4mpostVaccBI2	2.29	1.83	1.56	1.03	1.80	1.89	1.97	1.67
5mpostVaccS/5mpostVaccBI2	2.22	1.48	1.16	0.70	1.75	1.85	2.05	1.60
preVaccW	0.64	1.65	1.11	0.30	1.79	1.63	2.16	1.50
1mpostVaccW	2.26	1.63	1.29	1.44	1.77	2.10	2.06	1.94
2mpostVaccW	2.47	2.76	1.45	1.32	1.49	2.15	2.37	1.36
3mpostVaccW	2.44	1.57	1.77	1.63	1.86	2.17	2.39	1.56
4mpostVaccW	2.40	1.57	1.60	1.47	1.82	1.78	2.20	2.01
5mpostVaccW	2.44	1.56	1.39	1.32	1.71	2.27	2.32	1.92

	H16	**H17**	**H18**	**H19**	**H21**	**H22**	**H23**
preVaccS/preVaccBI1	1.20	1.86	1.46	2.16	0.98	2.29	1.95
preVaccBI2					1.10		
1mpostVaccS/1mpostVaccBI2	1.52	2.53	1.55	2.46	1.34	2.27	2.00
2mpostVaccS/2mpostVaccBI2	1.61	2.83	1.20	2.96	1.02	2.15	2.79
3mpostVaccS/3mpostVaccBI2	1.37	2.09		2.25	1.01	1.97	1.90
4mpostVaccS/4mpostVaccBI2	1.54	2.01	1.51	2.19	0.91	2.21	1.93
5mpostVaccS/5mpostVaccBI2	1.35	1.93	1.14	2.09	0.84	1.95	1.78
preVaccW/preW	0.41	1.42	1.13	2.28	1.03	1.49	1.99
1mpostVaccW	1.61	2.14	1.93	2.16	0.89	2.08	1.79
2mpostVaccW	1.46	2.08	1.45	2.22	1.15	1.96	2.04
3mpostVaccW	1.78	2.08	1.78	2.21	1.25	2.64	1.86
4mpostVaccW	1.68	2.03	1.55	2.42	1.14	2.61	2.26
5mpostVaccW	1.53	2.30	1.49	2.36	0.59	2.31	2.09

H: Horse number; OD: Optical density; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination;
preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
mpostVaccBI2: months after the second dose of the basic immunization (Group C only)
Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study;
Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI;
Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
Group D: No vaccination history and no vaccination as part of the study, natural exposure due to an
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 13 (Group A): Longitudinal EHV-4 antibody concentrations in horses vaccinated against EHV-4 with a basic immunization >4 years ago with regular boosters every 6 months including summer and winter during the study (n = 5)

	H12	**H13**	**H14**	**H22**	**H23**
preVaccS	2.06	2.00	1.76	2.29	1.95
1mpostVaccS	2.11	1.61	1.81	2.27	2.00
2mpostVaccS	2.85	2.90	1.64	2.15	2.79
3mpostVaccS	1.86	2.12	1.74	1.97	1.90
4mpostVaccS	1.89	1.97	1.67	2.21	1.93
5mpostVaccS	1.85	2.05	1.60	1.95	1.78
preVaccW	1.63	2.16	1.50	1.49	1.99
1mpostVaccW	2.10	2.06	1.94	2.08	1.79
2mpostVaccW	2.15	2.37	1.36	1.96	2.04
3mpostVaccW	2.17	2.39	1.56	2.64	1.86
4mpostVaccW	1.78	2.20	2.01	2.61	2.26
5mpostVaccW	2.27	2.32	1.92	2.31	2.09

H: Horse number; OD: Optical density: preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 14 (Group B): Longitudinal EHV-4 antibody concentrations in horses with no EHV-4 vaccination history which were vaccinated twice 6 months apart in summer and winter during the study (n = 4)

	H16	**H17**	**H18**	**H19**
preVaccS	1.20	1.86	1.46	2.16
1mpostVaccS	1.52	2.53	1.55	2.46
2mpostVaccS	1.61	2.83	1.20	2.96
3mpostVaccS	1.37	2.09		2.25
4mpostVaccS	1.54	2.01	1.51	2.19
5mpostVaccS	1.35	1.93	1.14	2.09
preVaccW	0.41	1.42	1.13	2.28
1mpostVaccW	1.61	2.14	1.93	2.16
2mpostVaccW	1.46	2.08	1.45	2.22
3mpostVaccW	1.78	2.08	1.78	2.21
4mpostVaccW	1.68	2.03	1.55	2.42
5mpostVaccW	1.53	2.30	1.49	2.36

H: Horse number; OD: Optical density: preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 15 (Group C): Longitudinal EHV-4 antibody concentrations in horses with no EHV-4 vacination history which received a basic immunization in summer (2 doses 4 weeks apart) followed by a booster vaccine 6 months later, in winter (n = 6)

	H7	**H8**	**H9**	**H10**	**H11**	**H21**
preVaccBI1	2.37	1.30	1.34	1.36	1.77	0.98
preVaccBI2	2.28	0.62	1.40	0.35	1.92	1.10
1mpostVaccBI2	3.03	2.92	2.07	1.90	2.62	1.34
2mpostVaccBI2	2.22	2.00	1.53	1.32	1.73	1.02
3mpostVaccBI2	2.24	1.80	1.62	1.07	1.67	1.01
4mpostVaccBI2	2.29	1.83	1.56	1.03	1.80	0.91
5mpostVaccBI2	2.22	1.48	1.16	0.70	1.75	0.84
preVaccW	0.64	1.65	1.11	0.30	1.79	1.03
1mpostVaccW	2.26	1.63	1.29	1.44	1.77	0.89
2mpostVaccW	2.47	2.76	1.45	1.32	1.49	1.15
3mpostVaccW	2.44	1.57	1.77	1.63	1.86	1.25
4mpostVaccW	2.40	1.57	1.60	1.47	1.82	1.14
5mpostVaccW	2.44	1.56	1.39	1.32	1.71	0.59

H: Horse number; OD: Optical density; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; mpostVaccBI2: months after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination;
positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

OD-Values corresponding to Fig. 16: Longitudinal EHV-4 antibody concentrations in control Group D (n = 5)

	H1	**H2**	**H3**	**H4**	**H6**
preVaccS	1.66	1.22	1.63	1.46	1.80
1mpostVaccS	1.42	1.62	1.92	1.34	1.64
2mpostVaccS	1.63	1.25	1.57	1.47	1.73
3mpostVaccS	1.74	1.01	1.55	1.31	1.84
4mpostVaccS	1.62	0.94	1.48	1.37	1.80
5mpostVaccS	2.00	1.19	1.58	1.38	1.73
preVaccW	Missing sample	Missing sample	Missing sample	Missing sample	Missing sample
1mpostVaccW	1.45	1.13	1.64	1.75	1.69
2mpostVaccW	1.85	0.94	1.79	1.41	1.69
3mpostVaccW	1.69	1.06	1.86	1.42	1.76
4mpostVaccW	1.50	1.40	1.75	1.52	1.68
5mpostVaccW	1.56	1.27	1.88	1.41	1.87

H: Horse number; OD: Optical density; preVaccS: pre summer vaccination; mpostVaccS: months post summer vaccination; preVaccW: pre winter vaccination; mpostVaccW: months post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred in the middle of timepoint 3mpostVaccS and 4mpostVaccS; positive results: > 0.2; negative results: < 0.1; non-interpretable results: 0.1 – 0.2

CT-Values corresponding to Fig. 17 (Group A-C): EHV-1 nasal shedding over 5 days in horses vaccinated against EHV-1/4 with different protocols (n = 15)

	H7	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**
preVaccS/preVaccBI1				*38.42*		*Missing sample*	*Missing sample*	*Missing sample*
1dpostVaccS/1dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
2dpostVaccS/2dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
3dpostVaccS/3dpostVaccBI1	45.00		45.00	37.85	45.00	Missing sample	Missing sample	Missing sample
4dpostVaccS/4dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
5dpostVaccS/5dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
preVaccBI2	*28.98*	*32.93*	*33.93*	*34.11*	*34.54*			
1dpostVaccBI2	45.00	45.00	45.00	45.00	45.00			
2dpostVaccBI2	45.00	45.00	45.00	45.00	36.34			
3dpostVaccBI2	45.00	45.00	45.00	45.00	45.00			
4dpostVaccBI2	45.00		45.00	45.00	45.00			
5dpostVaccBI2	43.70	35.82	45.00	45.00	45.00			
preVaccW	*45.00*	*27.56*	*31.95*	*27.92*	*31.80*	*43.19*	*34.47*	*45.00*
1dpostVaccW	45.00	45.00	45.00	45.00	43.19	45.00	45.00	45.00
2dpostVaccW	09.91	36.29	36.10	45.00	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	45.00	36.90	36.78	45.00	45.00	45.00	45.00
5dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00

	H16	H17	H18	H19	H21	H22	H23
preVaccS/preVaccBI1	*35.04*	*45.00*	*36.78*		*Missing sample*	*45.00*	*32.55*
1dpostVaccS/1dpostVaccBI1	45.00	45.00		45.00	Missing sample	45.00	36.74
2dpostVaccS/2dpostVaccBI1	45.00	38.91		45.00	Missing sample	45.00	45.00
3dpostVaccS/3dpostVaccBI1	45.00	45.00	45.00	45.00	Missing sample	45.00	45.00
4dpostVaccS/4dpostVaccBI1	45.00	45.00	45.00	45.00	Missing sample	45.00	45.00
5dpostVaccS/5dpostVaccBI1	45.00	45.00	45.00	45.00	Missing sample	45.00	45.00
preVaccBI2					*Missing sample*		
1dpostVaccBI2					Missing sample		
2dpostVaccBI2					Missing sample		
3dpostVaccBI2					Missing sample		
4dpostVaccBI2					Missing sample		
5dpostVaccBI2					Missing sample		
preVaccW	*34.81*	*36.72*	*45.00*	*36.90*	45.00	*45.00*	*45.00*
1dpostVaccW	45.00	37.06			45.00	45.00	45.00
2dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	38.75	45.00	45.00	45.00	45.00	45.00
5dpostVaccW	45.00		45.00	45.00	45.00	45.00	45.00

H: Horse number; CT: Cyclic Treshold; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination;
preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
dpostVaccBI1: days after the first dose of the basic immunization (Group C only)
dpostVaccBI2: days after the second dose of the basic immunization (Group C only)
Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study;
Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI;
Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 18 (Group A): Longitudinal EHV-1 nasal shedding in horses vaccinated against EHV-1 with a basic immunization >4 years ago with regular boosters every 6 months including summer and winter during the study (n = 5)

	H12	**H13**	**H14**	**H22**	**H23**
preVaccS	*Missing sample*	*Missing sample*	*Missing sample*	*45.00*	*32.55*
1dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	36.74
2dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
3dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
4dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
5dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
preVaccW	*43.19*	*34.47*	*45.00*	*45.00*	*45.00*
1dpostVaccW	45.00	45.00	45.00	45.00	45.00
2dpostVaccW	45.00	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	45.00	45.00	45.00	45.00
5dpostVaccW	45.00	45.00	45.00	45.00	45.00

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 19 (Group B): Longitudinal EHV-1 nasal shedding in horses with no vaccination history against EHV-1 and vaccinated twice 6 months apart in summer and winter during the study (n = 4)

	H16	**H17**	**H18**	**H19**
preVaccS	*35.04*	*45.00*	*36.78*	
1dpostVaccS	45.00	45.00		45.00
2dpostVaccS	45.00	38.91		45.00
3dpostVaccS	45.00	45.00	45.00	45.00
4dpostVaccS	45.00	45.00	45.00	45.00
5dpostVaccS	45.00	45.00	45.00	45.00
preVaccW	*34.81*	*36.72*	*45.00*	*36.90*
1dpostVaccW	45.00	37.06		
2dpostVaccW	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	38.75	45.00	45.00
5dpostVaccW	45.00		45.00	45.00

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 20 (Group C): Longitudinal EHV-1 nasal shedding in horses with no EHV-1 vacination history which received a basic immunization in summer (2 doses 4 weeks apart) followed by a booster vaccine 6 months later, in winter (n = 6)

	H7	**H8**	**H9**	**H10**	**H11**	**H21**
preVaccBI1				*38.42*		*Missing sample*
1dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample
2dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample
3dpostVaccBI1	45.00		45.00	37.85	45.00	Missing sample
4dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample
5dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample
preVaccBI2	*28.98*	*32.93*	*33.93*	*34.11*	*34.54*	*Missing sample*
1dpostVaccBI2	45.00	45.00	45.00	45.00	45.00	Missing sample
2dpostVaccBI2	45.00	45.00	45.00	45.00	36.34	Missing sample
3dpostVaccBI2	45.00	45.00	45.00	45.00	45.00	Missing sample
4dpostVaccBI2	45.00		45.00	45.00	45.00	Missing sample
5dpostVaccBI2	43.70	35.82	45.00	45.00	45.00	Missing sample
preVaccW	*45.00*	*27.56*	*31.95*	*27.92*	*31.80*	45.00
1dpostVaccW	45.00	45.00	45.00	45.00	43.19	45.00
2dpostVaccW	09.91	36.29	36.10	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	45.00	36.90	36.78	45.00	45.00
5dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00

H: Horse number; CT: Cyclic Treshold; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; dpostVaccBI2: days after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 21: Longitudinal EHV-1 nasal shedding in control Group D (n = 5)

	H1	**H2**	**H3**	**H4**	**H6**
preVaccS	45.00	45.00	45.00	45.00	45.00
1dpostVaccS	45.00	45.00	45.00	45.00	45.00
2dpostVaccS	45.00	45.00	45.00	45.00	45.00
3dpostVaccS	45.00	45.00	45.00	45.00	45.00
4dpostVaccS	45.00	45.00	45.00	45.00	45.00
5dpostVaccS	45.00	45.00	45.00	45.00	45.00
preVaccW	45.00	45.00	45.00	36.72	45.00
1dpostVaccW	45.00	45.00	45.00	45.00	45.00
2dpostVaccW	45.00	45.00	45.00	36.31	45.00
3dpostVaccW	35.83	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	45.00	45.00	45.00	45.00
5dpostVaccW	Missing sample	Missing sample	Missing sample	Missing sample	Missing sample

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period;
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 22 (Group A-C): EHV-4 nasal shedding over 5 days in horses vaccinated against EHV-1/4 with different protocols (n = 15)

	H7	**H8**	**H9**	**H10**	**H11**	**H12**	**H13**	**H14**
preVaccS/preVaccBI1				*34.14*		*Missing sample*	*Missing sample*	*Missing sample*
1dpostVaccS/1dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
2dpostVaccS/2dpostVaccBI1	45.00	45.00	45.00	45.00		Missing sample	Missing sample	Missing sample
3dpostVaccS/3dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
4dpostVaccS/4dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
5dpostVaccS/5dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample	Missing sample	Missing sample
preVaccBI2	*26.47*	*31.23*	*29.78*	*30.84*	*31.97*			
1dpostVaccBI2	45.00	45.00	45.00	45.00	45.00			
2dpostVaccBI2	45.00	45.00	45.00	45.00	45.00			
3dpostVaccBI2	45.00	45.00	45.00	45.00	45.00			
4dpostVaccBI2	45.00		45.00	45.00	45.00			
5dpostVaccBI2	35.85	35.21	45.00	45.00	36.21			
preVaccW	*45.00*	*24.98*	*29.27*	*24.58*	*29.30*	*33.67*	*43.90*	*45.00*
1dpostVaccW	45.00	37.83	45.00	34.92	33.67	45.00	45.00	*45.00*
2dpostVaccW	45.00	33.76	36.36	45.00	45.00	45.00	45.00	*45.00*
3dpostVaccW	45.00	34.91	45.00	45.00	45.00	45.00	45.00	*45.00*
4dpostVaccW	45.00	35.61	45.00	34.93	35.82	45.00	40.53	*45.00*
5dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00	45.00	*45.00*

	H16	**H17**	**H18**	**H19**	**H21**	**H22**	**H23**
preVaccS/preVaccBI1	*32.76*	*35.87*	*31.94*		*Missing sample*	*44.75*	*31.23*
1dpostVaccS/1dpostVaccBI1	45.00	45.00		45.00	Missing sample	45.00	45.00
2dpostVaccS/2dpostVaccBI1	45.00	45.00		45.00	Missing sample	45.00	45.00
3dpostVaccS/3dpostVaccBI1	45.00	45.00	45.00	45.00	Missing sample	45.00	45.00
4dpostVaccS/4dpostVaccBI1	45.00	35.53	45.00	36.54	Missing sample	45.00	45.00
5dpostVaccS/5dpostVaccBI1	45.00	45.00	45.00	45.00	Missing sample	45.00	45.00
preVaccBI2					*Missing sample*		
1dpostVaccBI2					Missing sample		
2dpostVaccBI2					Missing sample		
3dpostVaccBI2					Missing sample		
4dpostVaccBI2					Missing sample		
5dpostVaccBI2					Missing sample		
preVaccW	*32.63*	*33.37*	*45.00*	*33.41*	45.00	*38.74*	*45.00*
1dpostVaccW	45.00	45.00		45.00	45.00	45.00	45.00
2dpostVaccW	45.00	36.14	45.00	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	34.87	37.92	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	32.27	45.00	45.00	45.00	45.00	45.00
5dpostVaccW	45.00		45.00	45.00	45.00	45.00	45.00

H: Horse number; CT: Cyclic Treshold; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination;
preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
preVaccBI1: pre vaccination with the first dose of the basic immunization (Group C only);
preVacBI2: pre vaccination with the second dose of the basic immunization (Group C only);
dpostVaccBI1: days after the first dose of the basic immunization (Group C only)
dpostVaccBI2: days after the second dose of the basic immunization (Group C only)
Group A (H12, 13, 14, 22, 23) BI >4 years ago, q6months booster which was continued throughout the study;
Group B (H16, 17, 18, 19), no vaccination history, vaccinated two times, six months apart without prior BI;
Group C (H7, 8, 9, 10, 11, 21) no vaccination history, BI in summer followed by a booster after six months
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 23 (Group A): Longitudinal EHV-4 nasal shedding in horses vaccinated against EHV-4 with a basic immunization >4 years ago with regular boosters every 6 months including summer and winter during the study (n = 5)

	H12	**H13**	**H14**	**H22**	**H23**
preVaccS	*Missing sample*	*Missing sample*	*Missing sample*	*44.75*	*31.23*
1dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
2dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
3dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
4dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
5dpostVaccS	Missing sample	Missing sample	Missing sample	45.00	45.00
preVaccW	*33.67*	*43.90*	*45.00*	*38.74*	*45.00*
1dpostVaccW	45.00	45.00	*45.00*	45.00	45.00
2dpostVaccW	45.00	45.00	*45.00*	45.00	45.00
3dpostVaccW	45.00	45.00	*45.00*	45.00	45.00
4dpostVaccW	45.00	40.53	*45.00*	45.00	45.00
5dpostVaccW	45.00	45.00	*45.00*	45.00	45.00

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 24 (Group B): Longitudinal EHV-4 nasal shedding in horses with no vaccination history against EHV-4 and vaccinated twice 6 months apart in summer and winter during the study (n = 4)

	H16	**H17**	**H18**	**H19**
preVaccS	*32.76*	*35.87*	*31.94*	
1dpostVaccS	45.00	45.00		45.00
2dpostVaccS	45.00	45.00		45.00
3dpostVaccS	45.00	45.00	45.00	45.00
4dpostVaccS	45.00	35.53	45.00	36.54
5dpostVaccS	45.00	45.00	45.00	45.00
preVaccW	*32.63*	*33.37*	*45.00*	*33.41*
1dpostVaccW	45.00	45.00		45.00
2dpostVaccW	45.00	36.14	45.00	45.00
3dpostVaccW	45.00	34.87	37.92	45.00
4dpostVaccW	45.00	32.27	45.00	45.00
5dpostVaccW	45.00		45.00	45.00

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 25 (Group C): Longitudinal EHV-4 nasal shedding in horses with no EHV-4 vacination history which received a basic immunization in summer (2 doses 4 weeks apart) followed by a booster vaccine 6 months later, in winter (n = 6)

	H7	**H8**	**H9**	**H10**	**H11**	**H21**
preVaccBI1				*34.14*		*Missing sample*
1dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample
2dpostVaccBI1	45.00	45.00	45.00	45.00		Missing sample
3dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample
4dpostVaccBI1	45.00		45.00	45.00	45.00	Missing sample
5dpostVaccBI1	45.00	45.00	45.00	45.00	45.00	Missing sample
preVaccBI2	*26.47*	*31.23*	*29.78*	*30.84*	*31.97*	*Missing sample*
1dpostVaccBI2	45.00	45.00	45.00	45.00	45.00	Missing sample
2dpostVaccBI2	45.00	45.00	45.00	45.00	45.00	Missing sample
3dpostVaccBI2	45.00	45.00	45.00	45.00	45.00	Missing sample
4dpostVaccBI2	45.00		45.00	45.00	45.00	Missing sample
5dpostVaccBI2	35.85	35.21	45.00	45.00	36.21	Missing sample
preVaccW	*45.00*	*24.98*	*29.27*	*24.58*	*29.30*	45.00
1dpostVaccW	45.00	37.83	45.00	34.92	33.67	45.00
2dpostVaccW	45.00	33.76	36.36	45.00	45.00	45.00
3dpostVaccW	45.00	34.91	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	35.61	45.00	34.93	35.82	45.00
5dpostVaccW	45.00	45.00	45.00	45.00	45.00	45.00

H: Horse number; CT: Cyclic Treshold; BI: Basic immunization consisting of two doses given 4 weeks apart
preVaccBI1: pre vaccination with the first dose of the basic immunization; preVacBI2: pre vaccination with the second dose of the basic immunization; dpostVaccBI2: days after the second dose of the basic immunization; preVaccW: pre winter vaccination; mpostVaccW: days post winter vaccination;
Timepoints before each vaccination administration are written in italics
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

CT-Values corresponding to Fig. 26: Longitudinal EHV-4 nasal shedding in control Group D (n = 5)

	H1	**H2**	**H3**	**H4**	**H6**
preVaccS	45.00	45.00	45.00	45.00	45.00
1dpostVaccS	45.00	45.00	45.00	45.00	45.00
2dpostVaccS	45.00	45.00	34.99	45.00	45.00
3dpostVaccS	45.00	45.00	45.00	45.00	45.00
4dpostVaccS	45.00	45.00	45.00	45.00	45.00
5dpostVaccS	45.00	45.00	45.00	45.00	45.00
preVaccW	45.00	45.00	45.00	45.00	45.00
1dpostVaccW	45.00	45.00	45.00	45.00	45.00
2dpostVaccW	45.00	45.00	45.00	45.00	45.00
3dpostVaccW	45.00	45.00	45.00	45.00	45.00
4dpostVaccW	45.00	45.00	45.00	45.00	45.00
5dpostVaccW	Missing sample	Missing sample	Missing sample	Missing sample	Missing sample

H: Horse number; CT: Cyclic Treshold; preVaccS: pre summer vaccination; dpostVaccS: days post summer vaccination; preVaccW: pre winter vaccination; dpostVaccW: days post winter vaccination; No vaccination history and no vaccination as part of the study; natural exposure due to an outbreak occurred mid-terms between summer and winter sampling period;
The cut-off for a positive result is CT 45; positive results include CT-Values < 45

Acknowledgements

This work was supported by the “Foundation Pro Horse” (Stiftung Pro Pferd, project number PR 2019-01).

I would like to thank Prof. Dr. Colin Schwarzwald for giving me the opportunity to work at the institute.

Thanks a million to PD Dr. med. vet Angelika Schoster for her scientific support and great supervision. I appreciate that I could rely on you any time.

Thanks very much to Dr. med. vet Claudia Bachofen for organizing lab teaching and material, helping me with interpretation of lab results, as well as co-examining my dissertation.

Special thanks to Dr. Julia Lechmann and Isabelle Hardmeier from the Institute of Virology Zurich teaching me patently all required lab skills. You always had a sympathetic ear for me and I enjoyed learning from you. Additionally, thank you Isabelle for providing me part of your sampling material.

Many thanks to all members of the Institute of Virology Zurich, especially Debby, Charlotte, Karin, Marco and Jakub for helping me and having a pleasant atmosphere.

Thank-you very much, Julia van Spijk for organizing and collecting samples of the control Group. I was deeply grateful for your support.

I thank all horse owners and grooms from the bottom of my heart, providing their horses and supporting me in taking samples.

Finally I want to thank my family for all their support and encouragement during the whole process of this dissertation.

Curriculum Vitae

Vorname, Name	Laura Scheurer
Geburtsdatum	05.03.1989
Geburtsort	Bern (BE)
Nationalität	Schweizerin
Heimatort	Schnottwil (SO)
August/1996 – Juni/2003	Primarschule, Bäriswil/Hindelbank, Schweiz
September/2003 – Juni/2007	Gymnasium, Burgdorf, Schweiz
Juni 2007	Matura (Schwerpunkt Biologie und Chemie), Burgdorf, Schweiz
September 2007 – August 2008	Vorstudienpraktikum Hochschule für Landwirtschaft (HAFL), Zollikofen am Schweizerischen Nationalgestüt (SNG) und Biobetrieb «Gruth», Baselland
September 2008 – September 2009	Studium Agronomie HAFL, Zollikofen
September/2010 – Juli/2015	Studium der Veterinärmedizin, Universität Bern, Schweiz
Dezember 2015	Staatsexamen Veterinärmedizin., Universität Bern, Schweiz
März/2018 – Dezember/2020	Anfertigung der Dissertation unter Leitung von PD Dr. med. vet. Angelika Schoster am Departement für Pferde, Klinik für Pferdemedizin der Vetsuisse-Fakultät Universität Zürich, Vorsteher: Prof. Dr. med. vet. Colin Schwarzwald
Februar 2016 – Januar 2017	Internship an der ISME Pferdeklinik, Standort Avenches
Februar 2017 – Januar 2018	Internship an der Vetsuisse Fakultät Zürich, Schwerpunkt Pferdemedizin
Februar 2018 – Januar 2019	Assistenzstelle an der Pferdeklinik Dalchenhof, Brittnau
Seit Februar 2019	Tierärztin Grosstierpraxis Viehdoktor Zürcher Unterland in Teilzeitanstellung